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## **High mortality of mussels in northern Brittany – Evaluation of the involvement of pathogens, pathological conditions and pollutants**

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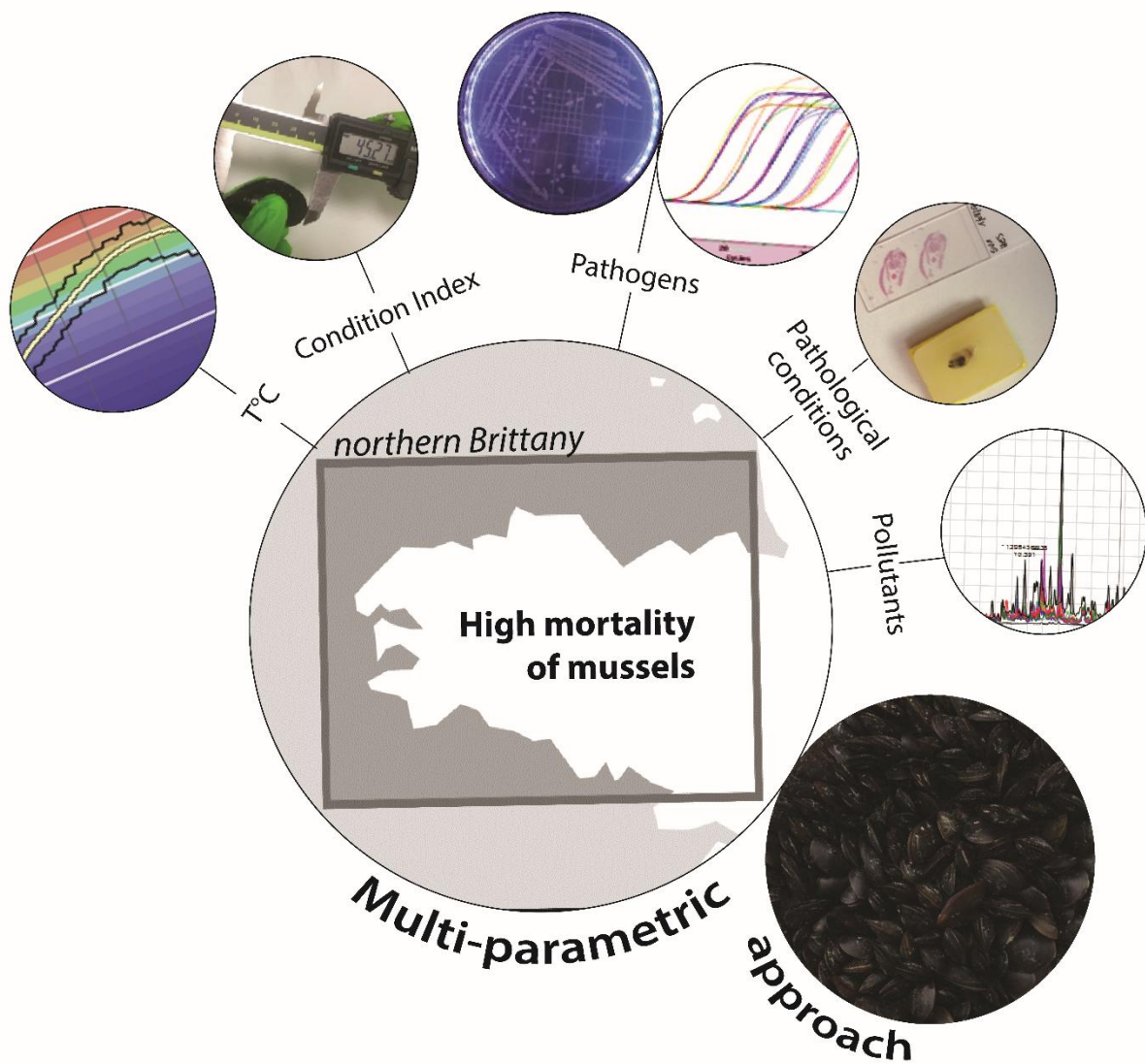


20 **Highlights**

- 21 • A multi-parametric approach was adopted to identify mortality causes in mussels.
- 22 • A ‘mortality window’ during the spring season was identified.
- 23 • Brest, Lannion and St. Brieuc (France) have different cumulative mussel mortality
- 24 rates.
- 25 • Haemocytic infiltrations and *Marteilia* sp. are parameters linked to mortality.
- 26 • There was no specific significant involvement of disseminated neoplasia or *Vibrio*.

27

28 **Graphical abstract**



29

30

31 **Abstract**

32 In 2014, a high and unusual mass mortality of mussels occurred in several important  
33 production areas along the French coasts of the Atlantic and English Channel. In the first  
34 quarter of 2016, mass mortalities hit farms on the west coast of the country once again. These  
35 heterogeneous mortality events elicited a multi-parametric study conducted during the 2017  
36 mussel season in three sites in northern Brittany (Brest, Lannion and St. Brieuc). The  
37 objective was to assess the health status of these mussels, follow mortality and attempt to  
38 identify potential causes of the abnormal high mortality of farmed mussels in northern  
39 Brittany. Brest was the most affected site with 70% cumulative mortality, then Lannion with  
40 40% and finally St. Brieuc with a normal value of 15%. We highlighted a temporal ‘mortality  
41 window’ that opened throughout the spring season, and concerned the sites affected by  
42 mortality of harmful parasites (including pathogenic bacteria), neoplasia, metal  
43 contamination, and tissue alterations. Likely, the combination of all these factors leads to a  
44 weakening of mussels that can cause death.

45 **Keywords:** *Mytilus edulis*; multi-parametric; neoplasia; *Vibrio splendidus*; *Marteilia*;  
46 hemocytic infiltration

47

48

## 49 **1. Introduction**

50 Mussels play a key role in the aquatic environment and are considered to be important  
51 ecosystem engineers by providing a suitable habitat for a species rich community  
52 (Buschbaum *et al.*, 2008). Due to their intense filtration activity, they modify aquatic habitat,  
53 making it more suitable for their own species and other for organisms (Borthagaray &  
54 Carranza, 2007). Mussels are also an important food source for many aquatic and terrestrial  
55 animals including humans, making them a product with high economic value. The blue  
56 mussel industry is important in Europe, a main producer of mussels globally, and France is  
57 second in Europe with a production of around 80,000 tons per year, most from aquaculture  
58 (FAO, 2018). In France, Brittany is a commercially important area for shellfish farming and is  
59 the second mussel producing region with 17,500 tons of *Mytilus* spp. produced in 2016  
60 (CNC, 2016).

61 In 2014, France was affected by high and unusual mass mortality of mussels, both  
62 juveniles and adults, affecting several important production areas along the French coast of  
63 the Atlantic and English Channel. Production losses reached 50 - 100% depending on the sites  
64 (Béchemin *et al.*, 2015; François *et al.*, 2015; Allain & Bernard, 2016; Lupo *et al.*, 2016). The  
65 phenomenon again affected farms on the west coast of France in the first quarter of 2016  
66 (FAO, 2016). Unlike mortality events in Pacific oyster (*Crassostrea gigas*), very few massive  
67 mortalities of *Mytilus* spp. occurred along the French coast until the winter of 2014 (Brienne,  
68 1964; Blateau, 1989; Guichard *et al.*, 2011).

69 One of the proposed causes of mortality is pathogenic bacteria in the genus *Vibrio* (Béchemin  
70 *et al.*, 2015). *Vibrios* are among the major bacterial pathogens of marine organisms and have  
71 been identified as pathogens of several bivalve molluscs (Beaz-Hidalgo *et al.*, 2010; Lemire  
72 *et al.*, 2015; Eggermont *et al.*, 2017). During mussel mortality events, different *Vibrio*  
73 *splendidus* strains were isolated from moribund mussels in France and were linked to  
74 mortality (Travers *et al.*, 2015; Ben Cheikh *et al.*, 2016). Of the sites studied, the Bay of

75 Brest, located at the extremity of the Brittany Peninsula, is one of the locations where mussels  
76 suffered massive mortality in 2014 and beyond. Estimated losses were 30% to 80%,  
77 depending on the year. *Vibrio* strains and pathogenic bacteria belonging to the Splendidus  
78 clade were identified and associated with these mortalities (François *et al.*, 2015; Lupo *et al.*,  
79 2016). Other researchers have suggested that the cause of mussel mortalities is disseminated  
80 neoplasia. This disease is well documented in marine bivalves all over the world (Peters,  
81 1988; Elston *et al.*, 1992; Landsberg, 1996; Carballal *et al.*, 2015) and, in some bivalve  
82 species including the mussel *Mytilus trossulus*, spreads by transmission of cancerous cells  
83 between individuals (Metzger *et al.*, 2015, 2016). Some scientists observed chromosomal  
84 abnormalities associated with neoplasia in *Mytilus trossulus* (González-Tizón *et al.*, 2000)  
85 while advanced neoplastic disease was associated with significant mortalities of *Mytilus* sp.  
86 (Moore *et al.*, 1991), including *M. trossulus* in Washington and Oregon (USA) and in British  
87 Columbia (Canada) (Elston *et al.*, 1992). In France, Benabdelmouna & Ledu (2016) and  
88 Benabdelmouna *et al.* (2018) presented evidence of the involvement of genomic  
89 abnormalities in mortality outbreaks in blue mussels and linked them to disseminated  
90 neoplasia. In addition, some cases of disseminated neoplasia in *Mytilus edulis* from Lannion  
91 (northern Brittany, France) were observed in mid-October 2016 by Burioli *et al.* (2017)  
92 during a mortality event.

93 Following heterogeneous mortality events of mussels that occurred in Brittany in the past  
94 years (Allain & Bernard, 2016; Bernard & Allain, 2017), the Regional Shellfish Committee of  
95 North Brittany (CRCBN) set up mortality monitoring in 2017 with coupled samplings over a  
96 period of 8 months. The initial focus was on the detection of neoplasia and on the  
97 involvement of a specific strain of *V. splendidus*. Nevertheless, it is known that the magnitude  
98 of mortality in bivalves can be modulated by several biotic factors, including the presence of  
99 pathogens (Carrasco *et al.*, 2015), and by abiotic factors such as chemical contaminants  
100 (Coles *et al.*, 1995; Pipe & Coles, 1995; Dailianis, 2010; Moschino *et al.*, 2016), water

101 temperature and animal conditioning (Almada-Villela *et al.*, 1982; Seed & Suchanek, 1992;  
102 Gosling, 1992, 2003). In an exploratory process, a field study conducted in partnership with  
103 mussel farmers was conducted *in situ*, wherein analyses, including biometry (condition  
104 index), histopathological examination, determination of bacterial flora, detection of bivalve  
105 mollusc pathogens and measurement of trace elements in mussel tissues, were carried out in  
106 three different sites in northern Brittany. Our objective of this *in situ* study was to identify  
107 potential causes responsible for the abnormal high mortality of farmed mussels in northern  
108 Brittany.

109

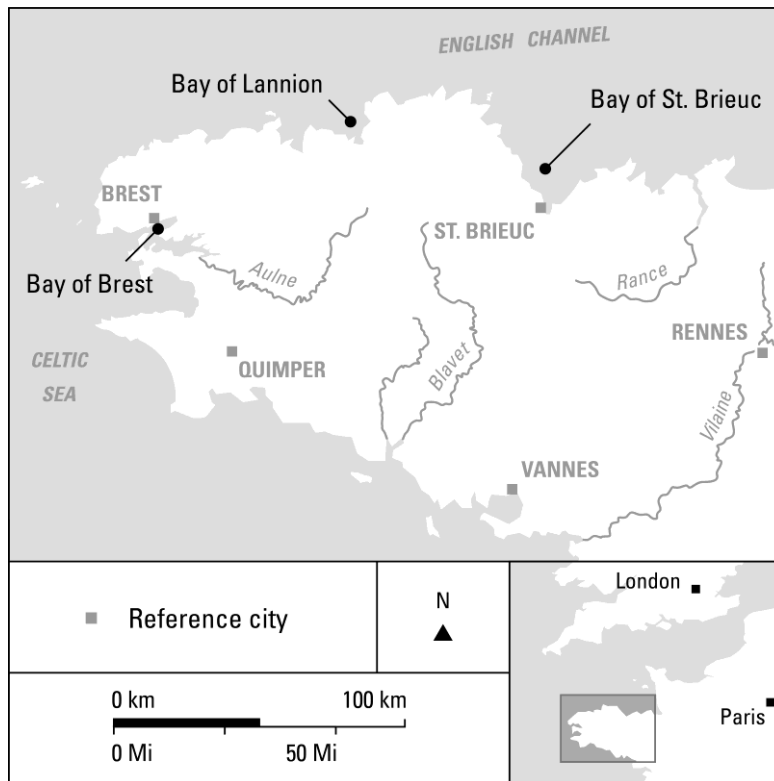
## 110 **2. Materials and methods**

111

### 112 ***2.1. Sampling sites and mussels***

113 Three primary mussel production sites in northern Brittany were selected for this study: the  
114 bays of Brest (Finistère, France), Lannion (Côtes-d'Armor, France) and St. Brieuc (Côtes-  
115 d'Armor, France) (Fig. 1). In each bay, one mussel farming company was involved in the  
116 study, representing approximately 8% of the mussel farmers in the Bay of St. Brieuc,  
117 approximately 25% of those located in the Bay of Brest, and the only farmer working in the  
118 Bay of Lannion. In each site, mussel farmers had introduced 7-mo-old mussels ( $23.51 \pm 4.62$   
119 mm in length) in the fall 2016 (Sept./Oct.) for their 2017 production season. These mussels  
120 were used in this study and were monitored until September 2017. The mussels of Brest  
121 originated in La Plaine-sur-mer (Loire-Atlantique, France), those of Lannion in Pénestin  
122 (Morbihan, France) and those of St. Brieuc in Noirmoutier (Vendée, France). The maximum  
123 distance between the origination sites is 60 km. The aim of this field study was to monitor and  
124 analyse mussels that were representative of those cultivated by the mussel farmers in their  
125 area, which depended exclusively on where mussel farmers obtained the spat.

126 The identification of species was determined from 400 mussels (50 per date and per site)  
127 sampled only for this purpose (Table 1). The determination of the species was performed by  
128 genotyping on a set of ancestry informative SNPs (Simon *et al.*, 2018).



129  
130 Figure 1: Location of the three sampling sites: the bays of Brest, Lannion and St. Brieuc.  
131

## 132 **2.2. Mortality and temperature monitoring**

133 In each site, 30 baskets of 100 mussels were prepared (Fig. 2A) and fixed on intertidal mussel  
134 stakes (“*bouchot*”; Fig. 2B) or subsurface longlines (for Lannion) at the beginning of the  
135 study. Every month, two baskets per site were collected and live mussels were counted for  
136 mortality monitoring. For Brest and St. Brieuc, in order to limit the time dedicated to counting  
137 during the tide, different baskets were counted each time; while for Lannion, because the tide  
138 is not a constraining factor, the same mussels were counted and put back into the baskets.  
139 Thus, each month, 200 mussels per site were counted to estimate mortality. Temperature  
140 sensors (HOBO Pendant<sup>®</sup>) were placed in the baskets containing mussels. These recorded the  
141 temperature around the animals (either water temperature or air temperature depending on  
142 tide) every 30 min.



143

144 Figure 2: Pictures of (A) the preparation of the baskets each containing 100 mussels and (B) the fixing of the  
145 baskets on the intertidal mussel stakes (“bouchot”). Photo credit: Regional Shellfish Committee of North  
146 Brittany (CRCBN)

147

### 148 ***2.3. Sampling for analysis***

149 Sampling for analysis was carried out with different frequency among sites: February, April,

150 May and September 2017 for Brest, and February and May 2017 for Lannion and St. Briec

151 (Table 1). At each sampling, 50 mussels per site were collected from baskets for histology,

152 and 20 additional mussels for biometry, molecular biology, bacteriological and chemical

153 analyses (performed on the same animals). The collected mussels were transported in thermal

154 bags to the laboratory and either analysed immediately or held in a refrigerator (4 °C)

155 overnight and analysed the next day.

Table 1. Information on study sites, sampling dates and number of mussels used for each analysis.

Site	Latitude	Longitude	Farming method	Sampling date				Species identification	Histological examination	Other analyses (biometry, bacteriological, molecular, and chemical analyses)
				Feb.	Apr.	May	Sept.			
Bay of Brest	48°20'22.5"N	4°19'48.4"W	<i>Bouchot</i>	27/02/2017	24/04/2017	30/05/2017	20/09/2017	50 per date (total=200)	50 per date (total=200)	20 per date (total=80)
Bay of Lannion	48°44'44.8"N	3°35'24.9"W	Longlines	24/02/2017		17/05/2017		50 per date (total=100)	50 per date (total=100)	20 per date (total=40)
Bay of St. Briec	48°33'07.6"N	2°38'55.6"W	<i>Bouchot</i>	27/02/2017		28/05/2017		50 per date (total=100)	50 per date (total=100)	20 per date (total=40)

## 1 **2.4. Biometry**

2 All mussel samples were individually weighed (wet flesh and shell separately after drainage  
3 of intervalvar water) with a precision scale ( $\pm 0.01$  g). A condition index (C.I.) was calculated  
4 as follows (Davenport & Chen, 1987):

$$C.I. = \frac{\text{Wet meat weight}}{\text{Shell weight}} \times 100$$

5

## 6 **2.5. Histological examination**

7 For dissection, shells were opened by cutting the adductor muscle and the soft tissues were  
8 removed. An approximately 5 mm thick transverse section of mussel tissue containing mantle  
9 lobes, visceral mass (gut, digestive gland) and gills was excised, placed into histological  
10 cassettes and transferred to Davidson's fixative for 48 h before being transferred to 70%  
11 ethanol. Fixed tissues were then dehydrated through an ascending ethanol series and  
12 embedded in paraffin wax. Thick sections, 5  $\mu\text{m}$ , were obtained using a rotary microtome and  
13 then stained with Harris' hematoxylin and eosin (HHE) (Howard *et al.*, 2004). Histological  
14 sections were examined for all symbionts, including phoresis, commensalism, parasitism and  
15 mutualism (Kinne, 1980), and pathological conditions under light microscopy. For each  
16 sampling date indicated in Table 1, 50 histological slides corresponding to the 50 sampled  
17 mussels were examined.

18

## 19 **2.6. Bacteriological analysis**

20 Analysis was conducted to compare the bacterial flora of mussels from each of the three sites  
21 on two different dates (February and May). For each of the six batches, tissues of five live  
22 mussels (to avoid the emergence and overexpression of opportunistic bacteria due to tissue  
23 degradation in dead animals) were pooled and mashed with scalpels and 200  $\mu\text{L}$  of Artificial  
24 Sterilized Sea Water (ASSW) was added to 50 mg of the homogenate. After stirring and pulse  
25 centrifugation to pellet cellular debris, ten-fold dilutions of supernatant were made and 100

26  $\mu\text{L}$  of 1:100 and 1:10,000 dilutions were sown on Zobell marine agar (Agar and ASSW  
27 enriched with 0.5% peptone, 0.1% yeast extract, and 0.01% ferric phosphate, pH 7.6;  
28 Oppenheimer & ZoBell, 1952) and incubated for 48 h at 22 °C. For each pool, 20  
29 predominant bacterial colonies with different phenotypes were re-isolated in order to verify  
30 their purity. DNA extraction was performed by heating a colony placed in 250  $\mu\text{L}$  of purified  
31 water (DNA/nuclease free-water) for 10 min at 95 °C. Successful extraction was confirmed  
32 by DNA quantification with NanoDrop<sup>TM</sup> 2000c spectrophotometer (ThermoFisher  
33 Scientific<sup>TM</sup>, Waltham, MA USA). Then, strains were characterized by molecular analysis  
34 (see below 2.6.1.).

35

## 36 **2.7. Molecular analyses**

37 The first step to determine the bacterial profile of the mussel batches consisted in  
38 discriminating between strains related to the *V. splendidus* clade (*V. splendidus*-related  
39 species) and other marine bacteria, as some vibrio strains belonging to this Splendidus clade  
40 are known to be pathogenic for molluscs. One TaqMan<sup>®</sup> real-time PCR, targeting the *16S*  
41 *rRNA* gene of *V. splendidus*-related strains (PCR1) (Oden *et al.*, 2016), was carried out on a  
42 Smart Cycler<sup>®</sup> (Cepheid, USA); the primers used for PCR1 were *SpFI*  
43 5'ATCATGGCTCAGATTGAACG3' and *SpRI* 5'CAATGGTTATCCCCACATC3' (Nasfi  
44 *et al.*, 2015) and the probe *SpProbe* 5'CCCATTAACGCACCCGAAGGATTG3'. The  
45 reaction volume of 25  $\mu\text{L}$  contained 12.5  $\mu\text{L}$  of Premix Ex Taq<sup>®</sup> 2 X Takara<sup>®</sup> (Lonza,  
46 Verviers, Belgium), 0.5  $\mu\text{L}$  of each primer (20 $\mu\text{M}$ ), 0.5  $\mu\text{L}$  of probe (10  $\mu\text{M}$ ), 9  $\mu\text{L}$  of purified  
47 water and 2  $\mu\text{L}$  of extracted DNA (replaced with 2  $\mu\text{L}$  of purified water in the negative  
48 control). The thermal cycling profile was 95°C for 10 s, followed by 40 cycles at 95 °C for 5 s  
49 and 62 °C for 30 s. When PCR1 was positive, a conventional PCR targeting the housekeeping  
50 genes *mreB* of *V. splendidus*-related strains (PCR2) was performed because it is the most  
51 discriminant gene for the identification of closely related strains among the Splendidus clade

52 (Oden *et al.*, 2016). The primer pair for PCR2 was *mreB*-F  
53 5'CTGGTGCTCGYGAGGTTTAC3' and *mreB*-R  
54 5'CCRTTYTCTGAKATATCAGAAGC3'. For colonies not identified as members of the  
55 Splendidus clade (PCR1 negative), another conventional PCR targeting the *16S rRNA* gene  
56 (PCR3) (Burioli *et al.*, 2018) was done; the primer pair for PCR3 was *16S27*-F  
57 5'AGAGTTTGATCMTGGCTCAG3' and *16S1492*-R 5'ACCTTGTTACGACTTCAC3'. For  
58 the conventional PCR, typical 25- $\mu$ L reaction mixtures contained 12.5  $\mu$ L of Premix Ex Taq<sup>®</sup>  
59 2 X Takara<sup>®</sup> (Lonza, Verviers, Belgium), 0.5  $\mu$ L of each primer (20  $\mu$ M), 9.5  $\mu$ L of purified  
60 water and 2  $\mu$ L of DNA template (replaced with 2  $\mu$ L of purified water in the negative  
61 control). Conventional PCR amplifications were carried out in a T100<sup>™</sup> Thermal Cycler  
62 (Bio-Rad, France) and the thermal program was as follows: 10 s at 95°C; 30 cycles for 10 s at  
63 95°C, 30 s at 55°C, 40 s at 72°C and a final extension of 3 min at 72°C. PCR products were  
64 then analysed with QIAxcel<sup>®</sup> Advanced System (Qiagen, Courtaboeuf, France) and those  
65 with the expected size were sent to Eurofins MWG Operon (Ebersberg, Germany) to be  
66 purified and sequenced. Species were identified using the National Center for Biotechnology  
67 Information (NCBI) Basic Local Alignment Search Tool (BLAST) with individual *16S*  
68 *rRNA* sequences, and *mreB* sequences were aligned using a multiple sequence alignment  
69 Multiple Sequence Comparison by Log-Expectation (MUSCLE). Phylogenetic analyses were  
70 performed in MEGA7 (Kumar *et al.*, 2016) using the Neighbor Joining method (Tamura *et*  
71 *al.*, 2013) and the maximum composite likelihood model with a bootstrap of 1000 replications;  
72 sequences of the *mreB* gene from 44 different reference bacterial strains from the Splendidus  
73 clade were used (see 2.1. in Oden *et al.*, 2016). *V. aestuarianus* 02/041, *V. ordalii* 12B09 and  
74 *V. penaeicida* AQ115, were provided from the Genomic of *Vibrio* Research Department  
75 (CNRS Roscoff, France) and used as the Splendidus clade outgroup.  
76 In parallel, the presence of known bivalve pathogens (*Bonamia* spp., *Haplosporidium nelsoni*,  
77 *Marteilia* sp., *Mikrocytos mackini*, *Nocardia crassostreae*, Ostreid Herpesvirus type 1

78 (OsHV-1), *V. aestuarianus*, *V. tubiashii*, *V. harveyi*, and *V. splendidus*) was investigated by  
79 PCR on DNA extracts from mussel tissues (Additional file 1).

80

## 81 **2.8. Chemical analyses of flesh**

82 The objective of chemical analyses was to assess if one site was more contaminated than  
83 others by one or more chemical compounds, which might explain why mortality was higher in  
84 one than in the others. For cost reasons, there were limitations in the number of samples that  
85 could be analysed. Because mortality began to increase in February, we chose to analyse and  
86 compare the three sites during that month rather than in May.

87 Presence of trace metal compounds (Pb, Hg, Cd, Co, Mo, Sn, Cr, Cu, Ni, Zn, Fe, Al, Mn and  
88 Ti) and non-metallic trace elements (As and Se) in mussel flesh was quantitatively determined  
89 by inductively coupled plasma-mass spectrometry (ICP-MS Triple Quad 8800, Agilent  
90 Technologies, Santa Clara, CA, USA) after wet digestion through high-quality grade mineral  
91 acids (HNO<sub>3</sub>, Analpure<sup>®</sup>, Analytika<sup>®</sup>, Prague, Czech Republic) and oxidants (H<sub>2</sub>O<sub>2</sub>,  
92 Normapur<sup>®</sup>, VWR international, Radnor, Pennsylvania, USA), following previously described  
93 protocols (Squadrone *et al.*, 2016). All analyses were calibrated against analytical standards.  
94 The limit of quantitation was 0.02 mg/kg for elements analysed by ICP-MS. All  
95 concentrations are given on a wet weight (w. w.) basis.

96

## 97 **2.9. Data analysis**

### 98 **2.9.1. Half-stock index**

99 To compare mortality phenomena over time, the half-stock index (Bernard *et al.*, 2018) is  
100 more suitable than using a cumulative mortality rate since it allows visualization of what is  
101 happening at a given time. Indeed, to compare different mortality episodes, it is preferable to  
102 consider the exponential aspect of population decrease. Thus, if  $\lambda$  is the mortality rate,  
103 supposed constant  $N(t)$  the population at  $t$  time, and  $N_0$  the population at the beginning of the

104 study, then:  $N(t) = N_0 e^{-\lambda t}$ . Between two successive counts in  $t_{i-1}$  and  $t_i$ , there is only one  $\lambda$   
105 which describes these two points:

$$\lambda = \frac{\ln(N_{i-1}) - \ln(N_i)}{t_i - t_{i-1}}$$

106 To obtain a more tangible index, the time (in days) needed to halve the population was used:  
107  $d_{0.5} = \ln(2) / \lambda$ . Therefore, this index corresponds to the number of days it takes to halve the  
108 population with the measured mortality rate, in a very similar way to half-life of radioactive  
109 elements. The lower this index, the higher the mortality rate and decrease of the stock. In the  
110 absence of mortality, this index tends towards infinity. For a better graphical representation, a  
111 maximum value was assigned to this index equal to 1500 (which corresponds to a loss of 15%  
112 over 1 year).

### 113 2.9.2 Statistical analysis

114 The half-stock index has been used to analyse and compare instantaneous mortality with  
115 different sampling intervals. It has also been used as the variable response to link the variation  
116 of mortality with the prevalence of pathological observations. This relationship was tested  
117 using a Spearman correlation test. Temperatures presented here have been smoothed in order  
118 to represent annual variation using generalized additive models with smoothing term with a  
119 moving window corresponding to one month.

120 Regarding biometry data, replicates were averaged, and the values were tested for normality  
121 (Shapiro). Then, a first ANOVA was used to compare the C.I. of Brest mussels at the four  
122 sampling dates, and a second to compare the C.I. of mussels from the three sites on both dates  
123 (February and March). When a significant difference was obtained, paired comparisons by  
124 Tukey's *post hoc* test were performed to identify which C.I. were different from the others.  
125 Statistical significance was accepted for  $*p < 0.05$ . The association between the C.I. and  
126 observed mortality was evaluated using a Pearson correlation test. Statistical analyses and  
127 graphical representations were performed by using R software, version 3.5.1 ([https://www.r-](https://www.r-project.org/)  
128 [project.org/](https://www.r-project.org/)) and 'dplyr', 'tidyr' and 'ggplot2' packages.

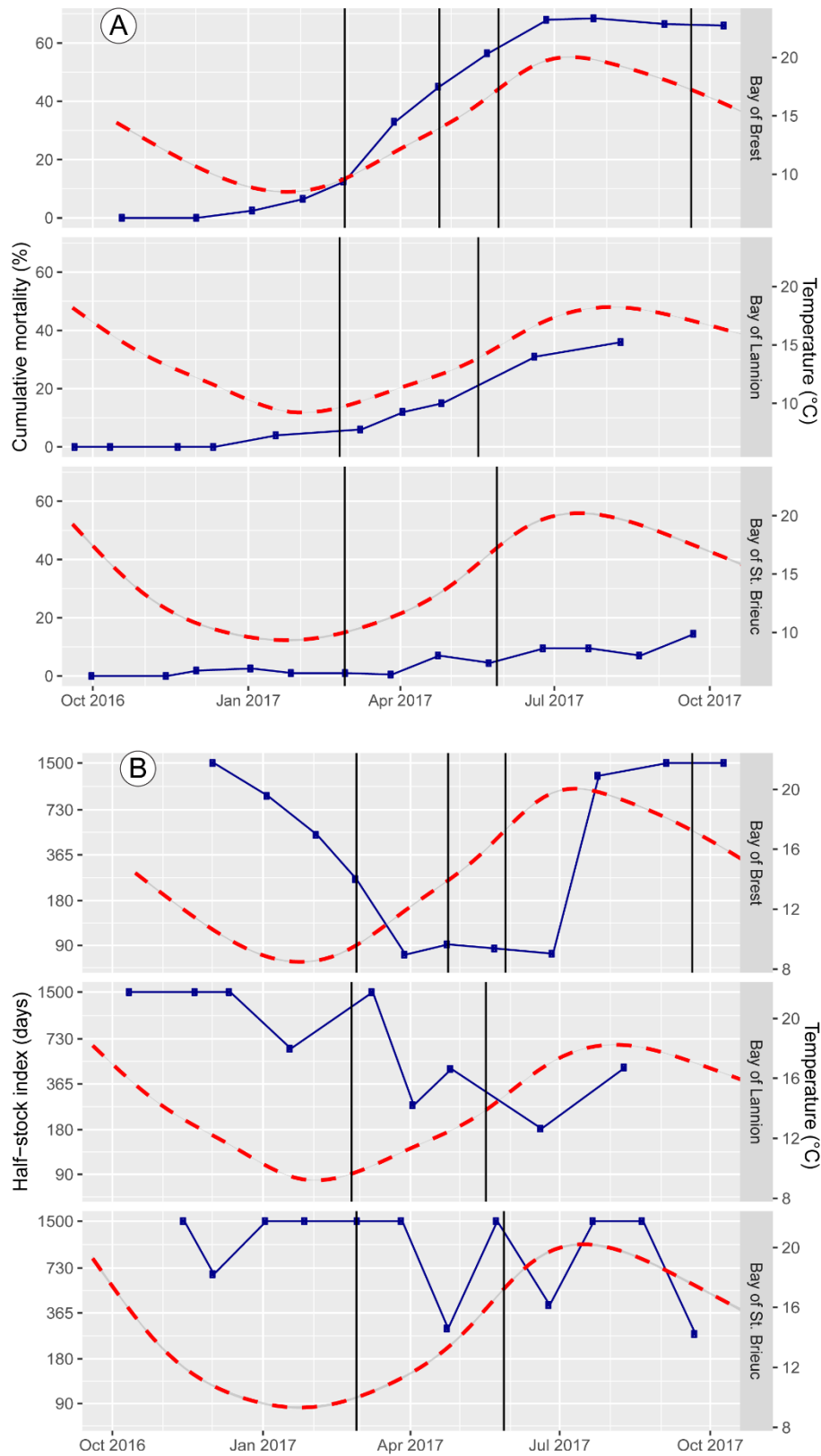
129 **3. Results**

130 **3.1. Mussel species, temperature monitoring and mortality**

131 For species identification, a dataset of 81 common markers was obtained (for more details,  
132 see Simon *et al*, 2019), which allowed identification of all mussels as the species *M. edulis*.

133 Regarding thermal profiles (Fig. 3), Lannion appears to be a rather temperate site compared to  
134 those of Brest and St. Brieuc, which showed wider temperature variation, especially higher  
135 temperatures in summer. From February to August, the temperature of Lannion rose from 9 to  
136 18 °C, that of St. Brieuc from 9 to 20 °C and that of Brest from 8.5 to 20 °C.

137 The first abnormal mortalities were observed at the very end of winter and early spring 2017  
138 (Fig. 3) in the most western site. Indeed, Brest was the most affected site as it suffered  
139 continuous mortality as illustrated by the half-stock index until mid-summer (Fig. 3B). The  
140 site had nearly 70% cumulative mortality at the end of the study (Fig. 3A). Lannion was less  
141 severely affected than Brest –but more than St. Brieuc– with a progressive decreasing trend in  
142 the half-stock index leading to a final cumulative mortality of almost 40%. St. Brieuc  
143 experienced some sporadic mortality peaks, but not considered abnormal. At the end of the  
144 study, a cumulative mortality of about 15% was obtained, which was a typical value for  
145 institutions (Council Directive 95/70/EC abrogated by 2006/88/CE) and mussel farmers.  
146 Furthermore, mortality decreased significantly or even stopped in mid-summer when  
147 temperatures exceeded 18 °C.



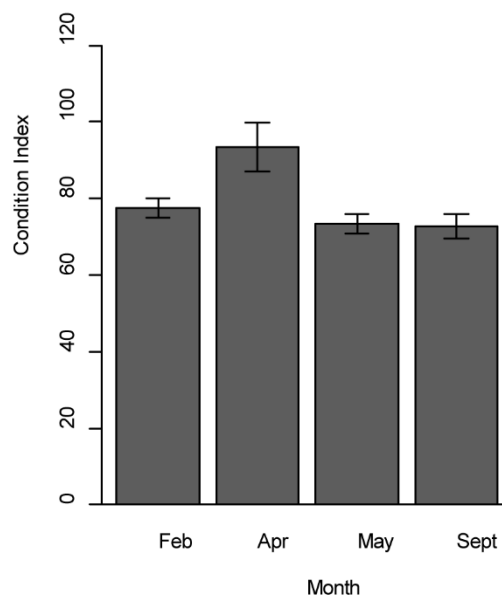
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149 Figure 3: Variation of temperature (broken line) and: (A) cumulative mortality (%) observed over the duration of  
 150 the study based on monthly mortality monitoring (continuous line with dots); (B) half-stock indices (days)  
 151 calculated monthly to visualize the occurrence of mortalities for each month (line with dots). The vertical black  
 152 lines correspond to the dates of sampling for analyses.

153

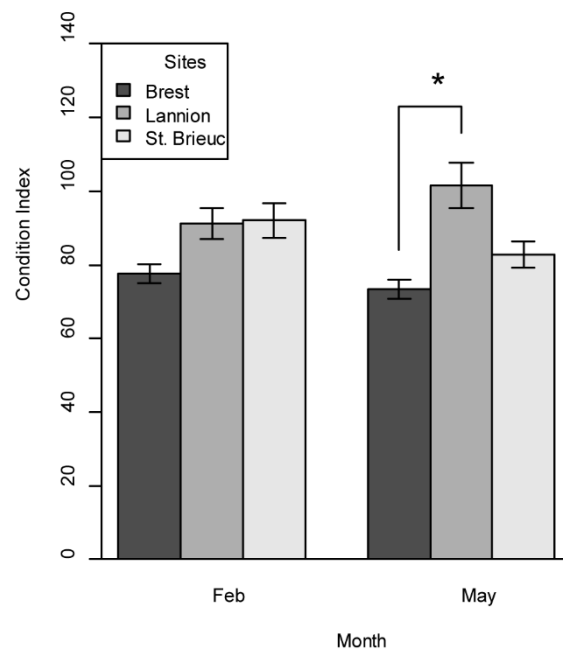
154 **3.2. Biometry**

155 When the C.I. of Brest mussels at the four sampling dates between February and September  
156 2017 were compared, despite an upward trend in the April index, no significant difference  
157 was observed (ANOVA,  $p > 0.05$ ) (Fig. 4). When the C.I. of mussels from the three sites on  
158 both dates (February and May 2017) were compared, no significant differences was observed  
159 in February but in May, the C.I. of mussels from Lannion were significantly higher than those  
160 of Brest mussels (ANOVA,  $p < 0.05$  and Tukey's *post hoc* test) (Fig. 5). There was no  
161 significant difference between the two C.I. in February and in May for the same site and no  
162 significant link appeared between observed mortality and C.I. (Pearson correlation,  $p > 0.05$ ).  
163



164

165 Figure 4: Evolution of the Condition Index (C.I.) of the Brest mussels on four dates (February, April, May and  
166 September 2017). Values are means  $\pm$  SEM (ANOVA,  $p > 0.05$ ).



167  
 168 Figure 5: Comparison of the Condition Indices (C.I.) of mussels from the 3 sites (Brest, Lannion, St. Brieuc) on  
 169 both dates (February and May 2017). Values are means  $\pm$  SEM (ANOVA, \* $p < 0.05$  and Tukey's *post hoc* test).  
 170

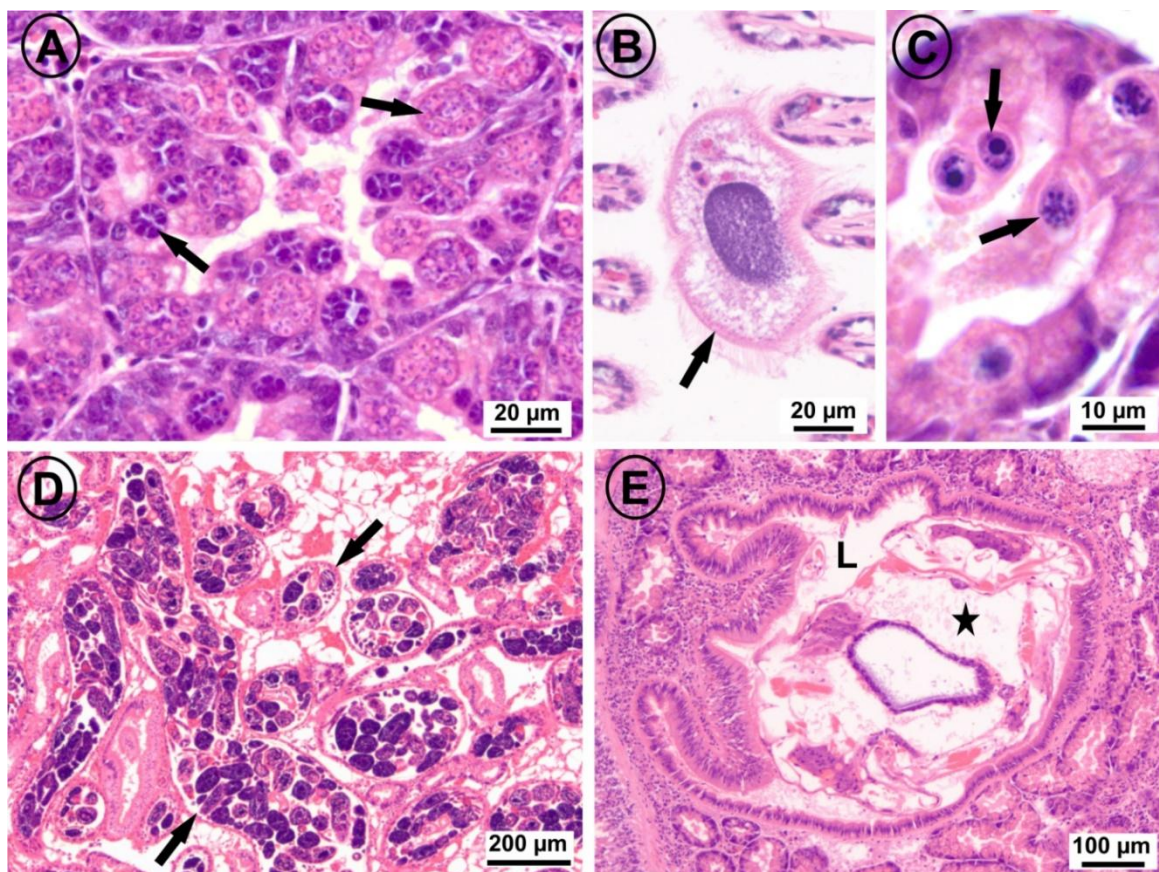
171 **3.3. Histological examination**

172 Histopathological examination showed the occurrence of various symbionts and pathological  
 173 conditions. Regarding symbionts, the different stages characterising parasites of the genus  
 174 *Marteilia* were observed in the stomach and digestive diverticula epithelia (Fig. 6A), from  
 175 primary cells enclosing some secondary cells in the stomach and digestive diverticula  
 176 epithelium to fully developed stages in digestive diverticula, in which secondary cells  
 177 included tertiary cells and had refringent granules in their cytoplasm. Occasionally, early  
 178 stages of *Marteilia* sp. were observed in the gills, associated with heavy inflammatory  
 179 response. Two types of ciliates were also observed, one type resembling *Ancistrum mytili* in  
 180 the gills (Fig. 6B) and intracellular ciliates in the epithelium of digestive tubules (Fig. 6C).  
 181 Trematode sporocysts enclosing developing cercariae were observed in the connective tissue  
 182 of the mantle and the visceral mass (Fig. 6D). *Mytilicola* sp. copepods were found in the  
 183 intestinal lumen (Fig. 6E). Regarding other pathological conditions, cases of heavy hemocytic  
 184 infiltration of the connective tissue of different organs were detected, but without any  
 185 identifiable inducing agent (Fig. 7A). The occurrence of large masses of hemocytes, mostly

186 granulocytes, surrounded by several layers of flattened, epithelioid cells, were also observed  
187 in the connective tissue of various organs (Fig. 7B). This type of inflammatory structure is  
188 usually called a granulocytoma and its occurrence was not linked to any detectable agent.  
189 Finally, cases of disseminated neoplasia, characterised by the infiltration of the connective  
190 tissue by abnormally large cells that also proliferate through the circulatory system (Fig. 7C)  
191 were observed. The nucleus of the abnormal cells was significantly larger than that of normal  
192 cells, with at least one patent nucleolus, showing frequent mitotic figures that indicated a high  
193 division ratio (Fig. 7D). The prevalence of symbionts and pathological conditions in each site  
194 is shown in Fig. 8. Regarding those with potentially the most serious consequences  
195 (granulocytomas, hemocytic infiltration, disseminated neoplasia, trematode sporocysts and  
196 *Marteilia*), Brest was the most affected site (Fig. 8A). Indeed, it was the only site in which 4  
197 out of 5 serious pathological conditions occurred in February (granulocytomas: 2.9%;  
198 hemocytic infiltration: 14.3%; trematode sporocysts: 11.4% and *Marteilia*: 22.9%) and in  
199 May (granulocytomas: 4.1%; hemocytic infiltration: 26.5%; trematode sporocysts: 2.0% and  
200 *Marteilia*: 22.5%). It was also the only site affected with *Marteilia* sp., which occurred over  
201 the entire study period and had a prevalence peak in April with 36% of individuals infected  
202 (Fig. 8B). In February, Lannion and St. Briec were affected by granulocytomas with similar  
203 prevalence (6.7% and 6.5%, respectively), twice that of Brest, and twice as many individuals  
204 showed trematode sporocysts at St. Briec (6.5%) than at Lannion (3.3%). In May, a low  
205 percentage of Lannion mussels had disseminated neoplasia (2.4%), while it was not detected  
206 in St. Briec. It is important to note that St. Briec had no sign of hemocytic infiltration,  
207 neither in February nor May. In contrast, St. Briec was the most affected by two less  
208 damaging symbionts, ciliates and *Mytilicola* sp., both in February and May. The prevalence of  
209 hemocytic infiltration increased in Brest from February to May, while it remained almost  
210 constant at Lannion. Figure 8B illustrated these observations for Brest; indeed, there was an  
211 increase in hemocytic infiltration and *Marteilia* between February and April, then a gradual

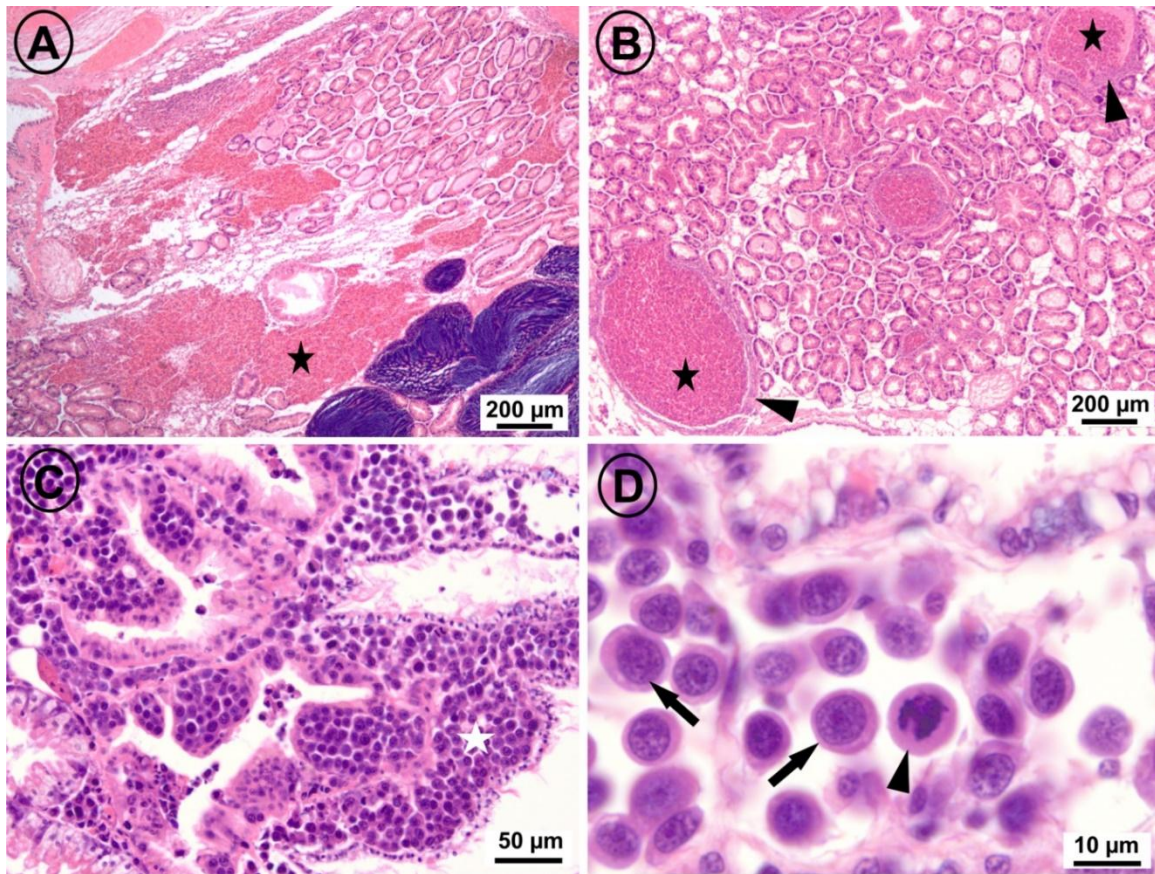
212 decrease from April to September. Furthermore, 18% of mussels from Brest showed  
213 disseminated neoplasia in April, while it was not found in February or May, and its  
214 prevalence was 2% in September. In Brest, the prevalence of most symbionts and the other  
215 pathological were highest in April. The prevalence of the symbionts and other pathological  
216 conditions showed an inverse relationship with the half-stock index. When testing the link  
217 between the prevalence of the different pathological conditions and the half-stock index  
218 (Spearman correlation test; Fig. 9), only hemocytic infiltration was significantly positively  
219 correlated to the intensity of mortality ( $p^* < 0.05$ ). Nevertheless, the p-value of *Marteilia* –  
220 equal to 0.08– was influenced by its absence in Lannion and St. Brieuc; in Brest, the higher  
221 the prevalence of *Marteilia*, the lower the half-stock index.

222



223

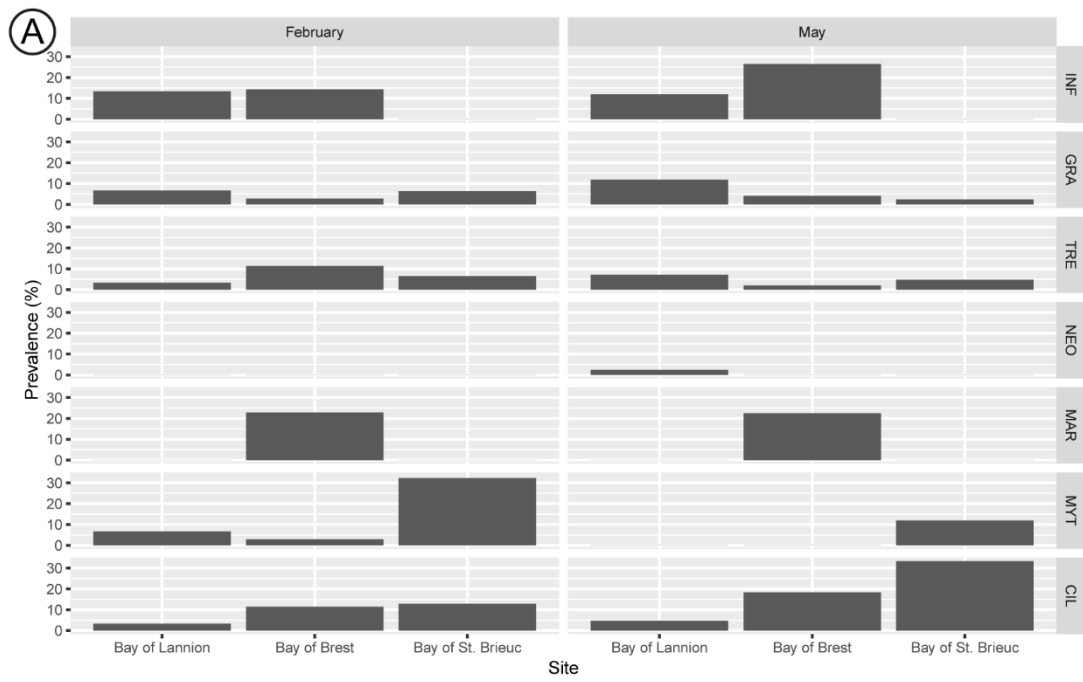
224 Figure 6: Micrographs of histological sections showing symbionts found in *Mytilus edulis*. A: section of the  
225 digestive gland showing different developing stages of *Marteilia* sp. (arrows) in the epithelium of digestive  
226 tubules. B: section through the gills showing a cell of the ciliate *Ancistrum mytili* (arrow). C: section through the  
227 digestive gland showing various intracytoplasmic ciliates (arrows) in the epithelium of a digestive tubule. D:  
228 section through the mantle showing trematode sporocysts (arrows) enclosing cercariae. E: section through the  
229 visceral mass showing a copepod *Mytilicola* sp. (star) in the intestinal lumen (L).



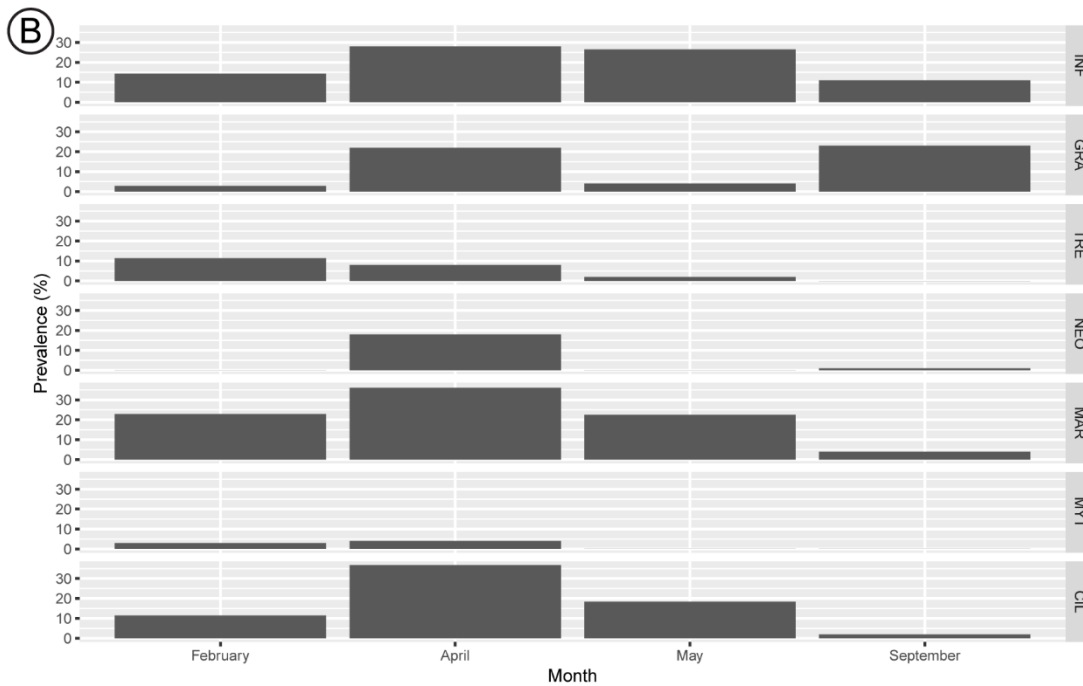
230

231 Figure 7: Micrographs of histological sections showing pathological conditions of *Mytilus edulis*. A: section  
 232 through the visceral mass showing heavy hemocytic infiltration (star) of the connective tissue. B: section through  
 233 the digestive gland showing granulocytomas consisting of large masses of hemocytes (stars) surrounded by  
 234 layers of flattened, epithelioid cells (arrowheads). C: section through the visceral mass showing the connective  
 235 tissue heavily infiltrated with masses of neoplastic cells (star). D: Higher magnification of the previous  
 236 micrograph showing abundant neoplastic cells (arrows) in a hemolymph sinus: a cell in mitotic process is  
 237 pointed out with an arrowhead.

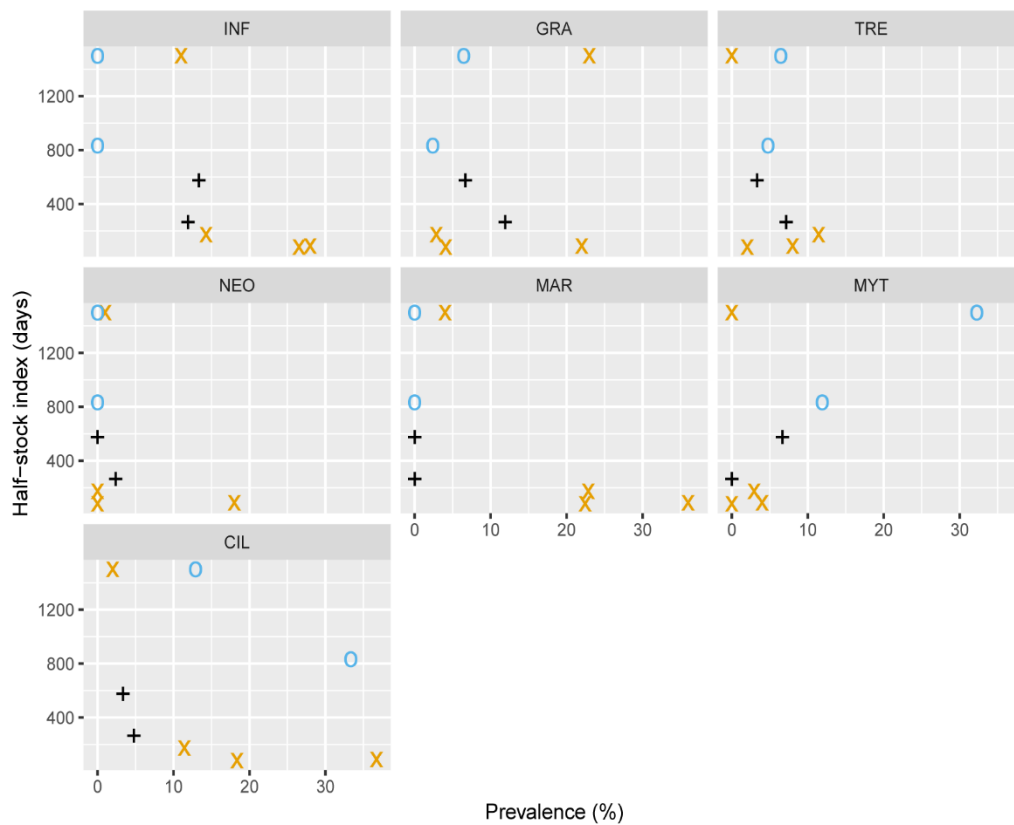
238



239



240 Figure 8: Prevalence (%) of the most remarkable histopathological observations: granulocytomas (GRA),  
 241 inflammation/haemocytic infiltration (INF), disseminated neoplasia (NEO), trematode sporocysts enclosing  
 242 cercariae (TRE), *Marteilia* sp. (MAR), *Mytilicola* sp.(MYT) and ciliates (CIL) identified (A) in the 3 bays  
 243 (Brest, Lannion, St. Brieuc) on both dates (February and May 2017); (B) in Brest on four dates (February, April,  
 244 May and September 2017).



245

246 Figure 9: Link between the prevalence (%) of symbionts and histopathological conditions and the half-stock index (days) for each site: Brest (letter x), Lannion (plus sign) and St. Brieuc (open circle). INF: haemocytic  
 247 infiltration; GRA: granulocytomas; TRE: trematode sporocysts enclosing cercariae; NEO: disseminated  
 248 neoplasia; MAR: *Marteilia* sp.; MYT: *Mytilicola* sp.; CIL: ciliates.

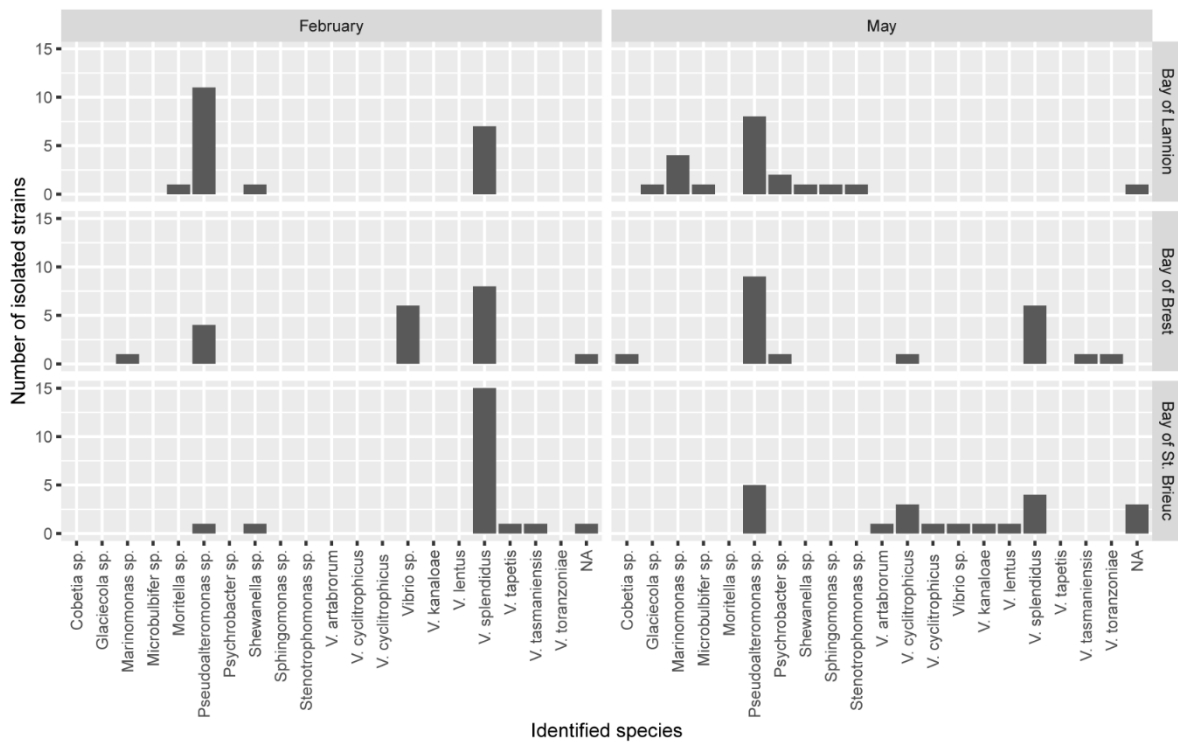
250

### 251 3.4. Molecular analyses

#### 252 3.4.1. Bacterial profiles

253 Following the identification of the 120 isolated bacterial strains from tissue culture, it was  
 254 noted that bacterial diversity was relatively higher in May than in February (Fig. 10). In  
 255 February, between four (Brest & Lannion) and five (St. Brieuc) different species per site were  
 256 present among the 20 strains isolated compared to seven (Brest & St. Brieuc) and nine  
 257 (Lannion) different species in May. However, despite this difference in diversity, two bacteria  
 258 remained dominant: *V. splendidus* and *Pseudoalteromonas* sp. *V. splendidus* prevalence in  
 259 February was 42% in Brest, 35% in Lannion and 80% in St. Brieuc; in May, prevalence was  
 260 30% in Brest, 0% in Lannion and 23% in St. Brieuc. In February *Pseudoalteromonas* sp.  
 261 prevalence was 21% in Brest, 55% in Lannion and 5% in St. Brieuc; while in May,  
 262 prevalence was 45% in Brest, 42% in Lannion and 29% in St. Brieuc. Among the 30 strains of

263 *V. splendidus* isolated in the three February batches –7 in Lannion, 15 in St. Brieuc and 8 in  
 264 Brest– all had a different *mreB* sequence, except for three strains found in St. Brieuc (1) and  
 265 in Brest (2), which had an identical sequence. When comparing bacterial profiles with the  
 266 occurrence of mortalities, no difference appeared; *V. splendidus* was found in high abundance  
 267 in almost all sites, as was *Pseudoalteromonas* sp. to a lesser extent, with or without  
 268 mortalities.  
 269



270  
 271 Figure 10: Bacterial profiles in samples from the 3 bays (Lannion, Brest, St. Brieuc) collected in February and  
 272 May 2017.  
 273

### 274 3.4.2. Presence or absence of known pathogens of bivalve molluscs

275 Among the four pools of DNA extracts from tissues of each of the nine batches we  
 276 investigated presence of 10 known pathogens, *Bonamia* spp., *Haplosporidium nelsoni*,  
 277 *Marteilia* sp., *Mikrocytos mackini*, *Nocardia crassostreae*, OsHV-1, *V. aestuarianus*, *V.*  
 278 *tubiashii*, *V. harveyi*, and *V. splendidus* belonging to Splendidus cluster. *V. splendidus* was  
 279 detected in all the batches from each site, while *Marteilia* sp. was recorded in all batches from

280 Brest (from February to September), which confirmed the histological results. All the other  
281 TaqMan<sup>®</sup> real-time PCR results were negative.

282

### 283 ***3.5. Chemical analyses of flesh***

284 Table 2 shows trace element concentrations in the mussel tissues and the maximum level  
285 (ML) set by the Commission Regulation ((EC) No. 1881/2006 amended by (EC) No.  
286 629/2008 and (EC) No. 420/2011) for regulated chemical compounds in the relevant  
287 foodstuffs category of bivalve molluscs. Comparing the three sites in February, mussels from  
288 St. Brieuc had concentrations of chromium (Cr), molybdenum (Mo), nickel (Ni) and titanium  
289 (Ti) overall 2-4x higher than those of Brest and Lannion. The Lannion site was distinguished  
290 by iron (Fe) and aluminum (Al) concentrations that were more than 2x lower than the other  
291 two sites. In contrast, lead (Pb) concentration was highest in Brest, more than 5x higher than  
292 that of the mussels from Lannion and St. Brieuc, and exceeding the ML. Regarding temporal  
293 variation of chemical compounds in Brest mussels, Pb concentration was similar in February  
294 and April with a twofold decrease in September; Fe and Al concentrations showed noticeable  
295 decrease over time. The highest concentrations were found in winter and a general decrease  
296 was observed throughout the study period.

297

299 Table 2. Trace element concentrations (mg/kg w.w.) in mussel tissues collected from three bays in northern  
 300 Brittany: Brest (BRE), Lannion (LAN) and St. Brieuc (STB), in February 2017, and in Brest in April and  
 301 September 2017. Limit of detection: < 0.002 mg/kg). *ML: Maximum Level set by the Commission*  
 302 *Regulation (EC) No. 1881/2006 amended by (EC) No. 629/2008 and (EC) No. 420/2011.*  
 303

<i>Chemical compounds</i>	<i>February</i>			<i>April</i>	<i>September</i>
	<b>STB</b>	<b>LAN</b>	<b>BRE</b>	<b>BRE</b>	<b>BRE</b>
<i>Cd (ML: 1.0)</i>	0.07	0.07	0.13	0.14	0.10
<i>Hg (ML: 0.5)</i>	0.01	0.02	0.04	0.03	0.02
<i>Pb (ML: 1.5)</i>	0.32	0.23	1.62	1.54	0.70
<i>Co</i>	0.15	0.09	0.21	0.12	0.07
<i>Cu</i>	1.42	1.35	1.43	1.36	1.59
<i>Cr</i>	3.80	1.49	0.99	0.54	0.09
<i>Fe</i>	175.98	62.28	157.48	85.65	34.10
<i>Mn</i>	3.15	1.79	2.69	1.81	1.22
<i>Mo</i>	0.84	0.37	0.23	1.32	0.14
<i>Ni</i>	2.31	1.00	0.65	0.49	0.12
<i>Zn</i>	13.7	15.84	15.85	18.73	12.32
<i>Ti</i>	5.90	1.57	1.91	0.95	0.38
<i>Sn</i>	<0.02	<0.02	<0.02	<0.02	0.19
<i>Al</i>	173.11	65.85	143.72	69.09	22.70
<i>Se</i>	1.28	1.48	1.39	1.04	0.85
<i>As</i>	3.16	3.99	3.15	6.42	3.41

304

305

#### 306 **4. Discussion**

307 An *in situ* study combining various analytical procedures was performed to explore  
 308 causes of mussel mass mortality. The experimental design for field work involved assuming  
 309 the culture procedures and using the mussel batches of the professional mussel farmers, taking  
 310 advantage of their activity to avoid any element or circumstance different from the common

311 practice that could distort the usual mussel performance through on-growing. This approach  
312 provided an accurate representation of what was occurring on the farms and was chosen as a  
313 first exploration assuming that more refined experimental designs eventually may be needed  
314 while taking advantage of the results of this first approach.

315

#### 316 ***4.1. Temperature, conditioning and mortality***

317 *Mytilus edulis* is known to resist extreme temperatures (Aarset, 1982; Seed &  
318 Suchanek, 1992; Almada-Villela *et al.*, 1982), however, the thermal profiles observed in our  
319 sites were not extreme or unusual. The narrower temperature range observed in Lannion was  
320 probably due to the farming method because mussels on subsurface longlines are always  
321 submerged and undergo less marked temperature variation than mussels on intertidal stakes.  
322 Results showed that a ‘mortality window’ opened in all sites during the spring season; similar  
323 observations also have been reported recently in France by other researchers (Benabdelmouna  
324 *et al.*, 2018; Dégremont *et al.*, 2019). This period corresponded to the spawning season of  
325 mussels. Gonad development usually begins in October/November, and by the end of the  
326 winter the gonads are ripe (Gosling, 2003). All energy accumulated during the quiescent  
327 period is used to fuel gametogenesis and finally spawning; thus, when mussels spawn, they  
328 are in poor condition during the remainder of the spring period, with low glycogen content  
329 (Najdek & Sapunar, 1987). Mussel growth is primarily influenced by the reproductive stage  
330 and food availability (Gosling, 1992) but growth capacities also have been linked to site and  
331 animal origin (Dickie *et al.*, 1984). The higher C.I. of mussels from Lannion in May could be  
332 explained by (i) their better growth capacities due to their origin, (ii) the greater trophic  
333 richness of the site, and (iii) the subtidal farming –which allows them continuous access to  
334 trophic resources. Indeed, Prou and Gouletquer (2002) showed that mussels grown on  
335 longlines had higher growth performance than mussels grown on *bouchot*. However, this did  
336 not prevent mortality. Primary production, and thus food availability, are reduced during

337 winter periods when seawater temperatures are below 10°C (Cloern, 1996) and mussels  
338 undergo long-time starvation periods (Harbach & Palm, 2018). *Mytilus* sp. can handle longer  
339 food restriction periods and maintain its shell size by using energy from its own tissue (Dare  
340 & Edwards, 1975; Riisgård *et al.*, 2014). However, no link between C.I. of mussels and  
341 mortalities obtained in site was found.

342 These various data and observations clearly illustrate that this transition period between  
343 winter and spring –corresponding to the spawning period– is stressful for mussels and  
344 decisive in their survival. Thus, spawning stress could contribute to mussel weakening and to  
345 an increased mortality rate to some extent. However, considering the very similar C.I.  
346 observed between the different sites studied –between those with high mortality and those  
347 with low mortality– this parameter alone cannot explain abnormal mortality.

348

#### 349 **4. 2 Mussel symbionts**

350 Villalba *et al.* (1997) classified mussel symbionts in three groups according to their  
351 pathogenicity. Among those identified in this study, ciliates are in the first group with  
352 unnoticeable pathogenic effects, *Mytilicola* sp. are in the second group that may damage the  
353 host, but are not lethal, and *Marteilia* sp. and trematodes are in the third group with  
354 potentially lethal effects.

355 Figueras *et al.* (1991) observed that, even at the highest densities of infestation, ciliates  
356 caused no detectable tissue damage in gills or in digestive tubules. More recent detailed  
357 studies of the intracellular ciliates in digestive tubules reported similar conclusions (Fichi *et*  
358 *al.* 2018). Our results are consistent with those reports, no associated inflammation or  
359 abnormal mortality was detected in St. Brieuç, the site most affected by ciliates. Regarding  
360 *Mytilicola* sp., Brienne (1964) and Blateau (1989) observed weight loss in French mussels  
361 infested with this copepod at the end of winter and early spring (breeding period); the  
362 mortality it caused was related to the decrease in mollusc vitality. Nevertheless, in most cases,

363 despite a high infestation intensity reported in English and Spanish mussels, it is evident that  
364 the host population can sustain the infestation indefinitely (Davey, 1989; Robledo *et al.*,  
365 1994). Again, our results were consistent because the highest prevalence of this copepod was  
366 recorded in St. Brieuc and no abnormal mortalities were observed over the period at this site;  
367 the mortality peak observed in May could be attributed to weakness due to the breeding  
368 season, which could be intensified by the presence of *Mytilicola* sp. as observed by Brienne  
369 (1964) and Blateau (1989).

370 Lauckner (1983) found that digenetic Trematoda were the most frequent and important  
371 metazoan parasites of bivalves. Indeed, trematode sporocysts and metacercaria were capable  
372 of causing a wide range of harmful consequences in their hosts (lesions, compression of  
373 tissues, castration, and deep weakness) that can lead to death when infestation is heavy  
374 (Robledo *et al.*, 1994; Laruelle *et al.*, 2002). Bakhmet *et al.* (2017) found a metabolic level  
375 supported by significant growth deficiency in parasitized *M. edulis*. This also was observed in  
376 Normandy (France) by Le Breton & Lubet (1992), as well as a variation in parasitism over  
377 time as we observed in Brest, and an increase in mortality between January and July.

378 No significant statistical link was established for *Marteilia* sp., but the p-value close to  
379 0.05 should be highlighted as it was entirely influenced by results from Brest. The presence of  
380 *Marteilia* sp. only in the most affected site should not be neglected, especially because it not  
381 only severely harms flat oysters, *Ostrea edulis*, but also *M. edulis* and *M. galloprovincialis*  
382 (Villalba *et al.* 1993; Villalba *et al.* 1997; Fuentes *et al.*, 1995; Arzul *et al.*, 2014). Indeed,  
383 several studies have shown that high mortality rates observed in certain mussel populations in  
384 Spain and France were positively correlated with the presence of this parasite (Villalba *et al.*,  
385 1993; Garcia *et al.*, 2005). Marteiliosis leads to the arrest of growth due to the degradation of  
386 digestive cells, loss of glycogen in tissues and resulting in considerable weight loss of the  
387 mollusc (Grizel & Tigé, 1973). This disease could explain the lower C.I. in Brest.  
388 Marteiliosis also causes a general weakening of the host, promoting opportunistic parasite

389 development (bacteria, ciliates) that could cause secondary infestations (Grizel, 1985). For  
390 Brest mussels, no significant changes were observed between the C.I. for weight calculated in  
391 February and those calculated in September. This is not typical because mussels should be  
392 fleshy at the end of summer and require accumulated energy reserves for gonadal  
393 development and winter survival. Previous studies in flat oysters showed that mortality due to  
394 *Marteilia* sp. was observed just after the breeding period during which the animal consumes a  
395 lot of energy. Marteiliosis also is known to cause hemocytic infiltration and occasionally  
396 granulocytomas, which cause the destruction of the pathogen but also of the host tissues  
397 (Villalba *et al.*, 1993). These effects were observed in Brest. The drastic decrease in  
398 prevalence observed in Brest between May and September could be explained by death of  
399 infected mussels. Histological examination does not allow discriminating between the  
400 congeneric species *Marteilia refringens* and *M. pararefringens*; although both species have  
401 been detected in mussels, *M. pararefringens* is more frequently reported (Kerr *et al.* 2018).  
402 The infected samples analysed with the real time PCR procedure showed infection with  
403 *Marteilia* sp.

404         Each of the symbionts and parasites detected in the study have a varying degree of  
405 negative effect on their hosts. A single non-lethal parasite may cause some damage but not  
406 death, but the cumulative effect of different non-lethal parasites may have an impact on the  
407 survival of the host. This is particularly true during a harsh season and at a key stage in the  
408 life cycle of the mussel. It is clear from our data that *Marteilia* was one of the major factors  
409 contributing to mortalities in Brest.

410

#### 411 ***4.3 Lesions and alteration: hemocytic infiltration, granulocytomas and neoplasia***

412         Some cases of hemocytic infiltration (positively correlated with mortality) and  
413 granulocytomas were not linked to a specific pathogen. It should be noted that abnormal is the  
414 most frequent alteration and this is considered to be an important biomarker of lesion and

415 inflammation in bivalves (Cuevas *et al.*, 2015). Cuevas *et al.* (2015) showed that mussels  
416 from the most impacted sites (by metallurgic and shipyard activities) endured the most  
417 significant deleterious effects showing inflammation. Also, Sheir & Handy (2010) established  
418 a potential link between hemocytic infiltration and lesions with the presence of xenobiotics.  
419 Lowe & Moore (1979) stated that granulocytomas reflect symptoms of long-term exposure to  
420 contaminants; the same observation was made about a potential correlation between neoplasia  
421 and water pollution because neoplastic disorders have been reported in bivalves collected  
422 from polluted areas (Lauckner, 1983; ICES, 2017).

423         In our study, pollution alone cannot explain the presence of granulocytomas. In May,  
424 they were more frequent in Lannion, the site with the lowest concentrations of chemical  
425 compounds. In addition, the highest prevalence of granulocytomas in Brest were observed in  
426 April and September while in these two months, the records of trace elements showed lower  
427 concentrations, more specially for lead. Finally, the prevalence of granulocytomas recorded in  
428 St. Brieuc was close to that of the other two sites and hemocytic infiltration and neoplasia  
429 were not recorded, while the concentration of all trace elements, except for lead, was higher in  
430 this site than in the others. Villalba *et al.* (2001) reported association of cockle, *Cerastoderma*  
431 *edule*, mortality with lesions resulting from heavy inflammatory reaction without a clearly  
432 identified cause, and with disseminated neoplasia. We observed inflammations with heavy  
433 hemocytic infiltrations correlated with mortality in Brest and Lannion, but not always  
434 associated with a specific cause. Disseminated neoplasia is a progressive disease insofar as  
435 neoplastic cells proliferate and replace normal hemocytes in circulation and could lead to  
436 death (Elston *et al.*, 1988; Ciocan & Sunila, 2005). Because hemocytes are cells with a key  
437 role in many physiological functions (digestion, excretion, nutrition, defense mechanisms;  
438 Cheng, 1984; Fisher, 1986, 1988), heavily affected mussels have reduced abilities and usually  
439 die (ICES, 2017). Indeed, mussels affected by disseminated neoplasia had, after the decrease  
440 in the number of normal circulating hemocytes, a weakened defense system and showed

441 reduced bacterial clearance (Kent *et al.*, 1989). Mass mortalities of various bivalve species  
442 have been associated with disseminated neoplasia (see reviews by Elston *et al.*, 1992;  
443 Carballal *et al.*, 2015). In France, Benabdelmouna & Ledu (2016) observed that genomic  
444 abnormalities, namely aneuploidy of the hemolymph cells, were significantly correlated with  
445 *Mytilus* sp. mortalities observed in 2015 in the French Atlantic coast. Furthermore,  
446 Benabdelmouna *et al.* (2018) linked the occurrence of a significant percentage of aneuploid  
447 hemolymph cells to disseminated neoplasia and concluded that this disease could be viewed  
448 as a major cause of morbidity and mortality for French mussels. Elston *et al.* (1988) followed  
449 the evolution of this disease over 4 months in *M. trossulus*; they reported a relatively long  
450 dynamic progression of the disease in the early stages (2-3 months) and then a quick  
451 evolution when disseminated neoplasia leads to rapid death. This is consistent with the  
452 mortality dynamics observed in our study and the absence, or possibly undetected early-stage  
453 cases of disseminated neoplasia in February. A relatively high prevalence of 18% was  
454 observed in Brest in April and therefore most certainly contributed to a percentage of  
455 mortality during this period and/or may also have been an aggravating factor with an already  
456 present pathology; but abnormal mortalities began in February with no sign of disseminated  
457 neoplasia being observed at that time. Lannion presents a similar pattern; in May few cases  
458 were found with a prevalence of 2.4% but no signs in February. We hypothesize that if  
459 histological observations on Lannion mussels had been made in April, a higher rate of  
460 neoplastic mussels would have been observed and may have played a role in the observed  
461 mortality percentage.

462 We showed that inflammation and hemocytic infiltration were correlated with mortality, but it  
463 was not always possible to link them to an identified cause. In Brest, pollution could have a  
464 role (see below) but for Lannion, the cause(s) remain unknown.

465

#### 466 ***4.4 Chemical contamination***

467 First, and in relation to what has been previously discussed, when food availability is  
468 low and competition between individuals is high, mussels increase their levels of water uptake  
469 through their gills. This leads to congestion and accumulation of useless substances in their  
470 tissues, including pollutants, which could result in increase of mortality rates (Dailianis,  
471 2010). It is known that anthropogenic compounds and heavy metals play roles in defence  
472 capacities of bivalve molluscs and could increase susceptibility to disease (Coles *et al.*, 1995;  
473 Pipe & Coles, 1995; Morley, 2010). In addition, the effects of environmental contaminants  
474 sometimes correspond to a direct toxic action on tissues or cells (Gagnaire *et al.*, 2004).

475 Some of the elements analyzed, Hg, As, Cd, Pb and Sn, have no known biological  
476 function and, except for Sn, are included in the list of the ten chemicals of major public health  
477 concern as part of International Programme on Chemical Safety (World Health Organisation,  
478 2010). These elements are natural trace components of the aquatic environment, but their  
479 levels increase due to agricultural, industrial and mining activities. Even low metal  
480 concentrations may threaten the health of aquatic and terrestrial organisms, humans included  
481 (Sarmiento *et al.*, 2011). Among all the metallic elements that Moschino *et al.*, (2016) have  
482 measured in mussel tissues (As, Cd, Cr, Pb, Al, Fe, Hg, Cu, Ni, Zn) during an *in situ* study  
483 over several years, a positive correlation between mussel mortality rate and Pb, Fe and Al was  
484 observed. Furthermore, the average concentrations they measured in the mussel tissues for  
485 these three elements were comparable with ours. It should also be noted that for Fe and Al,  
486 relatively similar high concentrations were found in St. Brieuc and Brest, while for lead, only  
487 mussels from Brest were above the threshold. Several biomarkers representative of the health  
488 status of the aquatic environment have been identified in mussels (Depledge, 1994; Dailianis,  
489 2010), and it was shown that mussels have lower defense mechanisms against metal oxidative  
490 challenge and toxicity than oysters. Indeed, Funes *et al.* (2006) have shown that activities of  
491 antioxidant enzymes are insufficient (compared to those observed in the Pacific oyster, *C.*  
492 *gigas*), which means that mussels are not sufficiently protected from the oxidative stress

493 associated with metal pollution. In addition, Viarengo *et al.* (1991) and Petrović *et al.*, (2004)  
494 observed seasonal variations in antioxidant responses related to physiological processes and  
495 showed inhibition of these defense mechanisms in winter related to the reproductive period  
496 and gonad resorption.

497 From the Chemical Contamination Observation Network of Ifremer (ROCCH), the  
498 Bay of Brest is known to be one of the sites most affected by lead contamination on the  
499 French coast for many decades (the first most contaminated on the west side of France) (Belin  
500 *et al.*, 2013). According to these authors and local authorities (*com. pers.*), the high Pb  
501 concentrations found in Brest mussels are mainly explained by the presence of silver lead  
502 mines around the Aulne river (surface area: 1842 km<sup>2</sup>) several kilometers upstream (at  
503 Poullaouën and Huelgoat, Finistère, France). The river provides more than 63% of the bay's  
504 freshwater supply (Auffret, 1983).

505 Lacroix *et al.* (2015) observed differences in physiological responses to contaminants  
506 between native Brest Bay and imported mussels. Non-natives were more sensitive and  
507 showed more sensitive biomarker responses. Therefore, it can be supposed that mussels  
508 caught in a more open and less contaminated marine area will be strongly impacted and  
509 stressed when they arrive in a more polluted area. Lacroix *et al.* (2017) also observed an  
510 altered physiological state, early spawning, in mussels in a polluted area (Bay of Brest)  
511 compared to those on the Atlantic coast of Brittany (under oceanic influence) suggesting that  
512 their health is compromised at this period. Therefore, the pollution in Brest is an additional  
513 deleterious factor to others previously discussed and could be an explanation for the heavy  
514 hemocytic infiltrations not found to be linked to a specific pathogen. However, this is  
515 probably not the case for Lannion, an unpolluted site.

516

#### 517 ***4.5 Bacterial profiles***

518 Species belonging to the genus *Pseudoalteromonas* are widely distributed in marine  
519 environments globally. Some studies have shown a positive role of *Pseudoalteromonas* sp.  
520 biofilms in the settlement of mussel, *Mytilus coruscus* larvae (Yang *et al.*, 2013; Li *et al.*,  
521 2014). On the other hand, Venkateswaran & Dohmoto (2000) identified a species of  
522 *Pseudoalteromonas* which play a natural antifouling role due to the products it excretes,  
523 which prevent fixation of mussel byssal threads. However, no pathogenic or lethal role has  
524 been demonstrated by this species on bivalves. Species of the genus *Pseudoalteromonas* and  
525 *Vibrio* are part of the normal microflora of temperate water marine animals and their  
526 proportion increases during the spoilage of the bivalves (Gram & Huss, 1996; Madigan *et al.*,  
527 2014). Also, Lokmer & Wegner (2015) showed that host-associated microbial communities  
528 are linked to abiotic and biotic factors.

529 The genus *Vibrio* includes a ubiquitous, diverse and abundant temperate coastal  
530 marine bacterial community (Thompson *et al.*, 2004). The species of the genus *Vibrio* show a  
531 very high diversity; more than 110 species have been identified, each with different  
532 relationships with their hosts, ranging from symbiosis to significant pathogenicity (Travers *et*  
533 *al.*, 2015). Among those that appear to be pathogenic to bivalves, species belonging to the  
534 Splendidus clade are systematically highlighted. For example, Béchemin *et al.* (2015)  
535 identified *V. splendidus*-related species in moribund mussels during mussel mass mortality  
536 outbreaks in summer 2014 in France; these isolates appeared to be capable of inducing  
537 mortality under laboratory conditions. Also, Ben Cheikh *et al.* (2016, 2017) observed that a  
538 pathogenic strain of *Vibrio splendidus* clade inhibits the immune response in *M. edulis* by  
539 altering hemocyte function and viability and causes hemocytic infiltration and  
540 granulocytomas. The high genotype diversity of the bacteria belonging to the Splendidus  
541 clade and the dynamic nature of microbial communities complicates considerably efforts to  
542 elucidate the role of *V. splendidus* clade bacteria in vibriosis (Kwan & Bolch, 2015). In  
543 addition, *Vibrio* studies are culture dependent and specialists themselves admit that they do

544 not know if the dynamics they observed in bacteria populations reflect physiological changes  
545 in a viable but non-cultivable state or fluctuations in density with environmental parameters  
546 (Thompson *et al.*, 2004). Knowledge of their role as animal pathogens and their mechanisms  
547 of action in pathogenesis has been limited. Indeed, Bruto *et al.* (2018) recently showed that,  
548 within the Splendidus clade, virulence represents an ancestral trait, but it has been lost from  
549 several populations. They identified two *loci* necessary for virulence and can now associate  
550 virulence in bivalves with one or many specific *V. splendidus* strains. Thus, simply finding  
551 some *V. splendidus* strains in mussels is no longer sufficient to incriminate them during a  
552 mortality episode. Accordingly, since *V. splendidus* was found in all sites, no matter the  
553 mortality rate, assessing whether these two virulence genes are present or not in our isolates  
554 would be highly interesting.

555         To explain abnormal mortalities, to identify a ‘biotic disease’ with one identifiable  
556 lethal pathogen (virus, bacteria or parasite) is desired, but there is evidence that surrounding  
557 conditions (abiotic factors like pollution, temperature and seasons), as well as the presence of  
558 multiple pathogens and parasites affecting the condition of animals are inseparable and cannot  
559 be considered individually. Because many factors could be involved, it is complicated to  
560 understand precisely mortality factors that have very high inter-site and inter-annual  
561 variability. Some factors can lead to gradual changes that progressively disrupt animal  
562 homeostasis while others can cause acute mortality. Dare (1976) concluded that mortality in  
563 mussel populations resulted from an interaction between several physical and biological  
564 factors. Similarly, Lokmer & Wegener (2015) argued that, in addition to the presence of  
565 pathogens, environmental factors have a strong impact on disease efficacy and mortality. In  
566 addition, it seems that genetics could also have an impact on mortality (Dickie *et al.*, 1984;  
567 Mallet *et al.*, 1987; Dégremont *et al.*, 2019).

568         Our experimental design, using different mussel stocks corresponding to different  
569 geographic sources in the different experimental sites, did not allow evaluating the influence

570 of the genetic background on the mussel mortality, that is to say if mussel stocks from  
571 different geographic sources would show different susceptibility to mortality outbreaks.  
572 Mussels on the French Atlantic coast have complex genetic structure (Bierne *et al.*, 2003; Fly  
573 *et al.*, 2015; Michalek *et al.*, 2016, Simon *et al.*, 2019).

574 Obviously, the possibility of missing an unknown and yet undetectable or uncultivable  
575 pathogen should not be excluded. Furthermore, a complex ‘pathosystem’ between two or  
576 more pathogens, as shown by De Lorgeril *et al.* (2018), may also exist and would require an  
577 integrative and holistic approach to be understood as recommended by these authors in the  
578 case of multi-factorial diseases.

579

## 580 **5. Conclusion**

581 This multi-parametric field study, carried out on commercially cultivated mussels, was  
582 a first approach to identifying potential causes of mortality in northern Brittany. We  
583 completed a first sorting and identified important factors for future experimental studies. In  
584 Brest, the presence of *Marteilia*, inflammatory lesions and pollution, with additional  
585 weakening factors such as the breeding period, explains part of the mortalities. In Lannion, no  
586 relevant parasites or pollution were found, although heavy inflammatory lesions were  
587 observed; thus, further research is required to explain those hemocytic infiltrations and to  
588 determine the cause(s) of the mortality observed in this site.

589

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599

600

601 **References**

- 602 Aarset, A. V. (1982). Freezing tolerance in intertidal invertebrates (a review). *Comparative*  
603 *Biochemistry and Physiology Part A: Physiology*, 73(4), 571–580.  
604 [https://doi.org/10.1016/0300-9629\(82\)90264-X](https://doi.org/10.1016/0300-9629(82)90264-X)
- 605 Allain, G., & Bernard, I. (2016). *Les mortalités de moules en 2014 et 2015 vues par les*  
606 *professionnels. Compte-rendu de la phase 1 : synthèse sur l'émergence, la propagation et*  
607 *l'installation des mortalités* [Technical Report]. Comité Régional de la Conchyliculture  
608 Bretagne nord. 28 pp.
- 609 Almada-Villela, P. C., Davenport, J., & Gruffydd, L. D. (1982). The effects of temperature on  
610 the shell growth of young *Mytilus edulis* L. *Journal of Experimental Marine Biology*  
611 *and Ecology*, 59(2–3), 275–288. [https://doi.org/10.1016/0022-0981\(82\)90121-6](https://doi.org/10.1016/0022-0981(82)90121-6)
- 612 Arzul, I., Chollet, B., Boyer, S., Bonnet, D., Gaillard, J., Baldi, Y., Robert, M., Joly, J. P.,  
613 Garcia, C., & Bouchouca, M. (2014). Contribution to the understanding of the cycle  
614 of the protozoan parasite *Marteilia refringens*. *Parasitology*, 141(02), 227–240.  
615 <https://doi.org/10.1017/S0031182013001418>
- 616 Auffret, G. A. (1983). *Dynamique sédimentaire de la Marge Continentale Celtique* [PhD  
617 Thesis]. Université de Bordeaux I. 391 pp. Retrieved from  
618 <https://archimer.ifremer.fr/doc/00034/14524/>
- 619 Bakhmet, I., Nikolaev, K., & Levakin, I. (2017). Effect of infection with Metacercariae of  
620 *Himasthla elongata* (Trematoda: Echinostomatidae) on cardiac activity and growth  
621 rate in blue mussels (*Mytilus edulis*) *in situ*. *Journal of Sea Research*, 123, 51–54.  
622 <https://doi.org/10.1016/j.seares.2017.03.012>
- 623 Beaz-Hidalgo, R., Balboa, S., Romalde, J. L., & Figueras, M. J. (2010). Diversity and  
624 pathogenicity of *Vibrio* species in cultured bivalve molluscs. *Environmental*  
625 *Microbiology Reports*, 2(1), 34–43. <https://doi.org/10.1111/j.1758-2229.2010.00135.x>
- 626 Béchemin, C., Soletchnik, P., Polsenaere, P., Le Moine, O., Pernet, F., Protat, M., Fuhrman,  
627 M., Quéré, C., Goultquer, S., Corporeau, C., Lapègue, S., Travers, M.-A., Morga, B.,  
628 Garrigues, M., Garcia, C., Haffner, P., Dubreuil, C., Faury, N., Baillon, L., Baud, J.-  
629 P., & Renault, T. (2015). Episodes de mortalité massive de moules bleues observés en  
630 2014 dans les Pertuis charentais. *Bulletin épidémiologique - Santé animale et*  
631 *alimentaion*, (67), 6–9.
- 632 Belin C., Claisse D., Daniel A., Fleury E., Miossec L., Piquet J.-C., Ropert M. Qualité du  
633 Milieu Marin Littoral- Synthèse Nationale de la - Surveillance 2013. Ifremer.  
634 <http://envlit.ifremer.fr/content/download/82798/598028/file/SyntheseNationaleBullSur>

- 636 Ben Cheikh, Y., Travers, M.-A., Morga, B., Godfrin, Y., Rioult, D., & Le Foll, F. (2016).  
637 First evidence for a *Vibrio* strain pathogenic to *Mytilus edulis* altering hemocyte  
638 immune capacities. *Developmental & Comparative Immunology*, 57, 107–119.  
639 <https://doi.org/10.1016/j.dci.2015.12.014>
- 640 Ben Cheikh, Y., Travers, M.-A., & Le Foll, F. (2017). Infection dynamics of a *V. splendidus*  
641 strain pathogenic to *Mytilus edulis*: *In vivo* and *in vitro* interactions with hemocytes.  
642 *Fish & Shellfish Immunology*, 70, 515–523. <https://doi.org/10.1016/j.fsi.2017.09.047>
- 643 Benabdelmouna, A., & Ledu, C. (2016). The mass mortality of blue mussels (*Mytilus* spp.)  
644 from the Atlantic coast of France is associated with heavy genomic abnormalities as  
645 evidenced by flow cytometry. *Journal of Invertebrate Pathology*, 138, 30–38.  
646 <https://doi.org/10.1016/j.jip.2016.06.001>
- 647 Benabdelmouna, A., Garcia, C., Ledu, C., Lamy, P., Maurouard, E., & Dégremont, L. (2018).  
648 Mortality investigation of *Mytilus edulis* and *Mytilus galloprovincialis* in France: An  
649 experimental survey under laboratory conditions. *Aquaculture*, 495, 831–841.  
650 <https://doi.org/10.1016/j.aquaculture.2018.06.075>
- 651 Bernard, I., & Allain, G. (2017). *Mortalités des moules en Bretagne nord : bilan des*  
652 *connaissances* [Technical Report]. Comité Régional de la Conchyliculture Bretagne  
653 nord. 37 pp.
- 654 Bernard, I., Charles, M., Allain, G., Burioli, E. A. V., Villalba, A., Le Foll, F., Deydier, Y.,  
655 Houssin, M. (2018). *Bilan de l'observatoire des mortalités de moules en Bretagne*  
656 *Nord pour la saison 2016-2017 et premiers éléments sur les organismes pathogènes*  
657 *présents* [Technical Report]. Comité Régional de la Conchyliculture Bretagne nord. 26  
658 pp.
- 659 Bierne, N., Borsa, P., Daguin, C., Jollivet, D., Viard, F., Bonhomme, F., David, P. (2003).  
660 Introgression patterns in the mosaic hybrid zone between *Mytilus edulis* and *M.*  
661 *galloprovincialis*. *Molecular Ecology*, 12, 447–461. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-294X.2003.01730.x)  
662 [294X.2003.01730.x](https://doi.org/10.1046/j.1365-294X.2003.01730.x)
- 663 Blateau, D. (1989). Expériences de traitement des moules (*M. edulis*) de bouchots de la baie  
664 du Mont Saint-Michel parasitées par *Mytilicola intestinalis* - Septembre 1987 et 1988  
665 [Technical Report]. 20 pp. Retrieved from Ifremer website:  
666 <https://archimer.ifremer.fr/doc/00000/1943/>
- 667 Borthagaray, A. I., & Carranza, A. (2007). Mussels as ecosystem engineers: Their  
668 contribution to species richness in a rocky littoral community. *Acta Oecologica*, 31(3),  
669 243–250. <https://doi.org/10.1016/j.actao.2006.10.008>

670 Brienne, H. (1964). Observations sur l'infestation des moules du pertuis breton par *Mytilicola*  
671 *intestinalis* Steuer. *Revue des Travaux de l'Institut des Pêches Maritimes*, 28(3), 205–  
672 230. Retrieved from Ifremer website: <https://archimer.ifremer.fr/doc/00000/4029/>

673 Bruto, M., Labreuche, Y., James, A., Piel, D., Chenivresse, S., Petton, B., Polz, M., & Le  
674 Roux, F. (2018). Ancestral gene acquisition as the key to virulence potential in  
675 environmental *Vibrio* populations. *The ISME Journal*, 12(12), 2954–2966.  
676 <https://doi.org/10.1038/s41396-018-0245-3>

677 Burioli, E. A. V., Charles, M., Bernard, I., Pitel, P. H., & Houssin, M. (2017). *First*  
678 *description of disseminated neoplasia in Mytilus edulis in northern Brittany (France)*  
679 *and development of a rapide diagnostic tool* [Scientific Poster]. Presented at the 2nd  
680 International Symposium on the Advances in Marine Mussel Research (AMMR),  
681 Sète, France. <https://doi.org/10.13140/RG.2.2.10832.20488>

682 Burioli, E. A. V., Varello, K., Lavazza, A., Bozzetta, E., Prearo, M., & Houssin, M. (2018). A  
683 novel divergent group of Ostreid herpesvirus 1  $\mu$ Var variants associated with a  
684 mortality event in Pacific oyster spat in Normandy (France) in 2016. *Journal of Fish*  
685 *Diseases*, 00, 1–11. <https://doi.org/10.1111/jfd.12883>

686 Buschbaum, C., Dittmann, S., Hong, J.-S., Hwang, I.-S., Strasser, M., Thiel, M., Valdivia, N.,  
687 Yoon, S.-P., & Reise, K. (2008). Mytilid mussels: global habitat engineers in coastal  
688 sediments. *Helgoland Marine Research*, 63(1), 47–58. [https://doi.org/10.1007/s10152-](https://doi.org/10.1007/s10152-008-0139-2)  
689 [008-0139-2](https://doi.org/10.1007/s10152-008-0139-2)

690 Carballal, M. J., Barber, B. J., Iglesias, D., & Villalba, A. (2015). Neoplastic diseases of  
691 marine bivalves. *Journal of Invertebrate Pathology*, 131, 83–106.  
692 <https://doi.org/10.1016/j.jip.2015.06.004>

693 Carrasco, N., Roozenburg, I., Voorbergen-Laarman, M., Itoh, N., & Engelsma, M. Y. (2013).  
694 Development of a real-time PCR for detection of the oyster pathogen *Nocardia*  
695 *crassostreae* based on its homogeneous 16S–23S rRNA intergenic spacer region.  
696 *Journal of Invertebrate Pathology*, 114(2), 120–127.  
697 <https://doi.org/10.1016/j.jip.2013.07.002>

698 Carrasco, N., Ford, S., & Anderson, R. (2015). Pathogens and disease processes in marine  
699 molluscs. *Journal of Invertebrate Pathology*, 131, 1–256.  
700 <https://doi.org/10.1016/j.jip.2015.09.005>

701 Cheng, T. C. (1984). A classification of molluscan hemocytes based on functional evidences.  
702 *In: Cheng, T. C. (Ed.) Invertebrate Blood. Comparative Pathobiology, Vol. 6, 111-*

703 146. Springer, Boston, MA. [https://doi.org/10.1007/978-1-4684-4766-8\\_5](https://doi.org/10.1007/978-1-4684-4766-8_5)

704 Ciocan, C., & Sunila, I. (2005). Disseminated neoplasia in blue mussels, *Mytilus*  
705 *galloprovincialis*, from the Black Sea, Romania. *Marine Pollution Bulletin*, 50(11),  
706 1335–1339. <https://doi.org/10.1016/j.marpolbul.2005.04.042>

707 Cloern, J. E. (1996). Phytoplankton bloom dynamics in coastal ecosystems: A review with  
708 some general lessons from sustained investigation of San Francisco Bay, California.  
709 *Reviews of Geophysics*, 34(2), 127–168. <https://doi.org/10.1029/96RG00986>

710 CNC, 2016. *La production française* [Online Access]. [http://www.cnc-france.com/La-](http://www.cnc-france.com/La-Production-francaise.aspx)  
711 [Production-francaise.aspx](http://www.cnc-france.com/La-Production-francaise.aspx)

712 Coles, J. A., Farley, S. R., & Pipe, R. K. (1995). Alteration of the immune response of the  
713 common marine mussel *Mytilus edulis* resulting from exposure to cadmium. *Diseases*  
714 *of Aquatic Organisms*, 22, 59–65. <https://doi.org/10.3354/dao022059>

715 Commission Regulation (EC) No. 1881/2006 [Online Access]. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN)  
716 [content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN)

717 Commission Regulation (EC) No. 629/2008 [Online Access]. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0629&qid=1548169909864&from=FR)  
718 [content/EN/TXT/PDF/?uri=CELEX:32008R0629&qid=1548169909864&from=FR](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0629&qid=1548169909864&from=FR)

719 Commission Regulation (EC) No. 420/2011 [Online Access]. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0420&from=EN)  
720 [content/EN/TXT/PDF/?uri=CELEX:32011R0420&from=EN](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0420&from=EN)

721 Council Directive 95/70/EC [Online Access]. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31995L0070&qid=1548169109196&from=FR)  
722 [content/EN/TXT/PDF/?uri=CELEX:31995L0070&qid=1548169109196&from=FR](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31995L0070&qid=1548169109196&from=FR)

723 Council Directive 2006/88/CE [Online Access]. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0088&from=FR)  
724 [content/EN/TXT/PDF/?uri=CELEX:32006L0088&from=FR](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0088&from=FR)

725 Cuevas, N., Zorita, I., Costa, P. M., Franco, J., & Larreta, J. (2015). Development of  
726 histopathological indices in the digestive gland and gonad of mussels: Integration with  
727 contamination levels and effects of confounding factors. *Aquatic Toxicology*, 162,  
728 152–164. <https://doi.org/10.1016/j.aquatox.2015.03.011>

729 Dailianis, S. (2010). Environmental impact of anthropogenic activities: The use of mussels as  
730 a reliable tool for monitoring marine pollution. In: MacGevin, L. E. (Ed.) *Mussels:*  
731 *Anatomy, Habitat and Environmental Impact*, Chap. 2, 43-72. Nova Science  
732 Publishers Inc, New York.

733 Dare, P. J., & Edwards, D. B. (1975). Seasonal changes in flesh weight and biochemical  
734 composition of mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales.  
735 *Journal of Experimental Marine Biology and Ecology*, 18(2), 89–97.  
736 [https://doi.org/10.1016/0022-0981\(75\)90066-0](https://doi.org/10.1016/0022-0981(75)90066-0)

737 Dare, P. J. (Ed.) (1976). *Settlement, growth and production of the mussel, Mytilus edulis L., in*

738 *Morecambe Bay, England*, 1-25. Her Majesty's Stationery Office, London.

739 Davenport, J., & Chen, X. (1987). A comparison of methods for the assessment of condition  
740 in the mussel (*Mytilus edulis* L.). *Journal of Molluscan Studies*, 53(3), 293–297.  
741 <https://doi.org/10.1093/mollus/53.3.293>

742 Davey, J. T. (1989). *Mytilicola intestinalis* (Copepoda: Cyclopoida): A ten year survey of  
743 infested mussels in a Cornish Estuary, 1978–1988. *Journal of the Marine Biological*  
744 *Association of the United Kingdom*, 69(04), 823–836.  
745 <https://doi.org/10.1017/S0025315400032197>

746 De Lorgeril, J., Lucasson, A., Petton, B., Toulza, E., Montagnani, C., Clerissi, C., Vidal-  
747 Dupiol, J., Chaparro, C., Galinier, R., Escoubas, J.-M., Haffner, P., Dégremont, L.,  
748 Charrière, G., Lafont, M., Delort, A., Vergnes, A., Chiarello, M., Faury, N., Rubio, T.,  
749 Leroy, M., Pérignon, A., Régler, D., Lorga, B., Alunno-Bruscia, M., Boudry, P., Le  
750 Roux, F., Destoumieux-Garzon, D., Gueguen, Y., & Mitta, G. (2018). Immune-  
751 suppression by OsHV-1 viral infection causes fatal bacteraemia in Pacific oysters.  
752 *Nature Communications*, 9(1). <https://doi.org/10.1038/s41467-018-06659-3>

753 Dégremont, L., Maurouard, E., Rabiller, M., Glize, P. (2019). Response to selection for  
754 increasing resistance to the spring mortality outbreaks in *Mytilus edulis* occurring in  
755 France since 2014. *Aquaculture*, 511.  
756 <https://doi.org/10.1016/j.aquaculture.2019.734269>

757 Dickie, L.M., Boudreau, P.R., Freeman, K.R. (1984). Influences of Stock and Site on Growth  
758 and Mortality in the Blue Mussel (*Mytilus edulis*). *Can. J. Fish. Aquat. Sci.* 41, 134–  
759 140. <https://doi.org/10.1139/f84-013>

760 Depledge, M. H., & Fossi, M. C. (1994). The role of biomarkers in environmental assessment  
761 (2). Invertebrates. *Ecotoxicology*, 3(3), 161–172. <https://doi.org/10.1007/BF00117081>

762 Eggermont, M., Bossier, P., Pande, G. S. J., Delahaut, V., Rayhan, A. M., Gupta, N., Islam, S.  
763 S., Yumo, E., Nevejan, N., Sorgeloos, P., Gomez-Gil, B., & Defoirdt, T. (2017).  
764 Isolation of Vibrionaceae from wild blue mussel (*Mytilus edulis*) adults and their  
765 impact on blue mussel larviculture. *FEMS Microbiology Ecology*, 93(4), 11.  
766 <https://doi.org/10.1093/femsec/fix039>

767 Elston, R. A., Kent, M. I., & Drum, A. S. (1988). Progression, lethality and remission of  
768 hemic neoplasia in the bay mussel *Mytilus edulis*. *Diseases of Aquatic Organisms*, 4,  
769 135–142. <https://doi.org/10.3354/dao004135>

770 Elston, R. A., Moore, J. D., & Brooks, K. (1992). Disseminated neoplasia of bivalve mollusc.  
771 *Reviews in Aquatic Science*, 6, 405–466.

772 FAO, 2016. Demand for European mussels within the EU on the decline. *In: GLOBEFISH -*

773 *Information and analysis on world fish trade* [Online Access]. <http://www.fao.org/in->  
774 [action/globefish/market-reports/resource-detail/en/c/450826/](http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/450826/)

775 FAO, 2018. Fisheries and aquaculture software. FishStatJ - software for fishery statistical  
776 time series. In: *FAO Fisheries and Aquaculture Department* [Online Access].  
777 <http://www.fao.org/fishery/>

778 Fichi, G., Carboni, S., Bron, J. E., Ireland, J., Leaver, M. J., & Paladini, G. (2018).  
779 Characterisation of the intracellular protozoan MPX in Scottish mussels, *Mytilus*  
780 *edulis* Linnaeus, 1758. *Journal of Invertebrate Pathology*, 153, 99–108.  
781 <https://doi.org/10.1016/j.jip.2018.02.022>

782 Figueras, A. J., Jardon, C. F., & Caldas, J. R. (1991). Diseases and parasites of rafted mussels  
783 (*Mytilus galloprovincialis* Lmk): preliminary results. *Aquaculture*, 99(1–2), 17–33.  
784 [https://doi.org/10.1016/0044-8486\(91\)90285-F](https://doi.org/10.1016/0044-8486(91)90285-F)

785 Fisher, W. S. (1986). Structure and Functions of Oyster Hemocytes. In: Bréhelin, M. (Ed.)  
786 *Immunity in Invertebrates*, Chap. 3, 25-35. Springer-Verlag, Berlin, Heidelberg.

787 Fisher, W. S. (1988). Environmental influence on host response: Environmental influence on  
788 bivalve hemocyte function. *American Fisheries Society Special Publication*, 18, 225-  
789 237.

790 Fly, E.K., Hilbish, T.J., Wethey, D.S., Rognstad, R.L. (2015). Physiology and biogeography:  
791 The response of European mussels (*Mytilus* spp.) to climate change. *American*  
792 *Malacological Bulletin*, 33, 136–149. <https://doi.org/10.4003/006.033.0111>

793 François, C., Garcia, C., Lupo, C., Travers, M.A., Morga, B., Tourbiez, D., Faury, N.,  
794 Haffner, P., Serpin, D., Dubreuil, C., Chollet, B., Baillon, L., Lapegue, S., Renault, T.  
795 (2015). *Bilan 2014 du réseau Repamo - Réseau national de surveillance de la santé*  
796 *des mollusques marins* (p. 60) [Technical Report].

797 Fuentes, J., Villalba, A., Zapata, C., & Alvarez, G. (1995). Effects of stock and culture  
798 environment on infections by *Marteilia refringens* and *Mytilicola intestinalis* in the  
799 mussel *Mytilus galloprovincialis* cultured in Galicia (NW Spain). *Diseases of Aquatic*  
800 *Organisms*, 21, 221–226. <https://doi.org/10.3354/dao021221>

801 Funes, V., Alhama, J., Navas, J. I., López-Barea, J., & Peinado, J. (2006). Ecotoxicological  
802 effects of metal pollution in two mollusc species from the Spanish South Atlantic  
803 littoral. *Environmental Pollution*, 139(2), 214–223.  
804 <https://doi.org/10.1016/j.envpol.2005.05.016>

805 Gagnaire, B., Thomas-Guyon, H., & Renault, T. (2004). *In vitro* effects of cadmium and  
806 mercury on Pacific oyster, *Crassostrea gigas* (Thunberg), haemocytes. *Fish &*  
807 *Shellfish Immunology*, 16(4), 501–512. <https://doi.org/10.1016/j.fsi.2003.08.007>

808 Garcia, C., Arzul, I., Chollet, B., Francois, C., Goubet, A., Joly, J.-P., Miossec, L., Robert,  
809 M., Cuvelier, N., Lefebvre, A., Le Gagneur, E., Ropert, M., Mouillard, G., Gerla, D.,  
810 Le Gal, D., Rocher, G., Langlade, A., Bedier, E., Nourry, M., Martin, J.-L.,  
811 Constantini, L., Masson, J.-C., & Martin, A.-G. (2005). *Bilan 2004 du réseau*  
812 *REPAMO - Réseau national de surveillance zoosanitaire des mollusques marins* (p.  
813 68) [Technical Report]. Retrieved from Ifremer website:  
814 <https://archimer.ifremer.fr/doc/00086/19702/>

815 González-Tizón, A., Martínez-Lage, A., Ausio, J., & Méndez, J. (2000). Polyploidy in a  
816 natural population of mussel, *Mytilus trossulus*. *Genome*, 43(2), 409–411.  
817 <https://doi.org/10.1139/g99-138>

818 Gosling, E. (Ed.) (1992). *The mussel Mytilus: ecology, physiology, genetics and culture.*  
819 *Developments in Aquaculture and Fisheries Science, Vol. 25*, 590 pp. Elsevier  
820 Science, Amsterdam.

821 Gosling, E. (Ed.) (2003). *Bivalve Molluscs: Biology, Ecology and Culture*, 454 pp. Fishing  
822 News Books, Oxford.

823 Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products.  
824 *International Journal of Food Microbiology*, 33(1), 121–137.  
825 [https://doi.org/10.1016/0168-1605\(96\)01134-8](https://doi.org/10.1016/0168-1605(96)01134-8)

826 Grizel, H., & Tigé, G. (1973). *La maladie de la glande digestive d'Ostrea edulis Linne. Actes*  
827 *CIEM, CM 1973/K:13* [Symposium Deed]. Presented at the « Comité des crustacés,  
828 coquillages et benthos ». Retrieved from <https://archimer.ifremer.fr/doc/00000/5929/>

829 Grizel, H. (1985). *Etude des récentes epizooties de l'huître plate Ostrea edulis Linne et de*  
830 *leur impact sur l'ostreiculture bretonne* [PhD Thesis]. Université des Sciences et  
831 Techniques du Languedoc. Retrieved from  
832 <https://archimer.ifremer.fr/doc/00000/2581/>

833 Guichard, B., François, C., Joly, J.-P., Garcia, C., Saulnier, D., Pépin, J.-F., Arzul, I., Omnes,  
834 E., Tourbiez, D., Chollet, B., Faury, N., Haffner, P., Robert, M., Renault, T. (2011).  
835 *Bilan 2010 du réseau Repamo - Réseau national de surveillance de la santé des*  
836 *mollusques marins* (p. 22) [Technical Report]. Retrieved from Ifremer website:  
837 <https://archimer.ifremer.fr/doc/00059/17050/14599.pdf>

838 Harbach, H., & Palm, H. W. (2018). Development of general condition and flesh water  
839 content of long-time starved *Mytilus edulis*-like under experimental conditions.  
840 *Aquaculture, Aquarium, Conservation and Legislation Bioflux*, 11(2), 8.

841 Howard, D. W., Lewis, E. J., Keller, B. J., & Smith, C. S. (2004). *Histological techniques for*  
842 *marine bivalve mollusks and crustaceans, 2nd edition*, 5218 pp. National Oceanic and

843 Atmospheric Administration Technical Memorandum National Ocean Service  
844 National Center for Coastal Ocean Science, 5, Oxford.

845 ICES, 2017. Originals by Alderman, D. J., Green, M. (No. 11) and Balouet, G. (No. 12)  
846 Revised by Tristan Renault and Susan Ford. 2017. *Disseminated neoplasms in*  
847 *bivalves*. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish,  
848 No. 67, 6 pp. <http://doi.org/10.17895/ices.pub.2098>

849 Kent, M. L., Elston, R. A., Wilkinson, M. T., & Drum, A. S. (1989). Impaired defense  
850 mechanisms in bay mussels, *Mytilus edulis*, with hemic neoplasia. *Journal of*  
851 *Invertebrate Pathology*, 53(3), 378–386. [https://doi.org/10.1016/0022-2011\(89\)90103-](https://doi.org/10.1016/0022-2011(89)90103-1)  
852 [1](https://doi.org/10.1016/0022-2011(89)90103-1)

853 Kerr, R., Ward, G. M., Stentiford, G. D., Alfjorden, A., Mortensen, S., Bignell, J. P., Feist, S.  
854 W., Villalba, A., Carballal, M. J., Cao, A., Arzul, I., Ryder, D., & Bass, D. (2018).  
855 *Marteilia refringens* and *Marteilia pararefringens* sp. nov. are distinct parasites of  
856 bivalves and have different European distributions. *Parasitology*, 145(11), 1483–1492.  
857 <https://doi.org/10.1017/S003118201800063X>

858 Kinne, O. (Ed.) (1980). *Diseases of marine animals. Vol. 1: General Aspects, Protozoa to*  
859 *Gastropoda*, 466 pp. John Wiley & Sons, Brisbane.

860 Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics  
861 Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7),  
862 1870–1874. <https://doi.org/10.1093/molbev/msw054>

863 Kwan, T., & Bolch, C. (2015). Genetic diversity of culturable *Vibrio* in an Australian blue  
864 mussel *Mytilus galloprovincialis* hatchery. *Diseases of Aquatic Organisms*, 116(1),  
865 37–46. <https://doi.org/10.3354/dao02905>

866 Lacroix, C., Richard, G., Segueineau, C., Guyomarch, J., Moraga, D., & Auffret, M. (2015).  
867 Active and passive biomonitoring suggest metabolic adaptation in blue mussels  
868 (*Mytilus* spp.) chronically exposed to a moderate contamination in Brest harbor  
869 (France). *Aquatic Toxicology*, 162, 126–137.  
870 <https://doi.org/10.1016/j.aquatox.2015.03.008>

871 Lacroix, C., Duvieilbourg, E., Guillou, N., Guyomarch, J., Bassoulet, C., Moraga, D., &  
872 Auffret, M. (2017). Seasonal monitoring of blue mussel (*Mytilus* spp.) populations in a  
873 harbor area: A focus on responses to environmental factors and chronic contamination.  
874 *Marine Environmental Research*, 129, 24–35.  
875 <https://doi.org/10.1016/j.marenvres.2017.04.008>

876 Landsberg, J. H. (1996). Neoplasia and biotoxins in bivalves: is there a connection? *Journal*  
877 *of Shellfish Research*, 15(2), 203–230.

- 878 Laruelle, F., Molloy, D. P., & Roitman, V. A. (2002). Histological analysis of trematodes in  
879 *Dreissena polymorpha*: their location, pathogenicity, and distinguishing  
880 morphological characteristics. *Journal of Parasitology*, 88(5), 856–863.  
881 [https://doi.org/10.1645/0022-3395\(2002\)088\[0856:HAOTID\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2002)088[0856:HAOTID]2.0.CO;2)
- 882 Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In: Kinne, O. (Ed.) *Diseases of Marine*  
883 *Animals, Vol. 2: Introduction, Bivalvia to Scaphopoda*, 477-961. John Wiley & Sons,  
884 Brisbane.
- 885 Le Breton, J., & Lubet, P. (1992). *Resultats d'une intervention sur une parasitose a*  
886 *Proctoeces maculatus (Trematoda, Digenea) affectant la mytiliculture de l'ouest*  
887 *Cotentin* [Technical Report] 14, 107–118. Retrieved from  
888 <https://archimer.ifremer.fr/doc/00000/922/>
- 889 Lemire, A., Goudenège, D., Versigny, T., Petton, B., Calteau, A., Labreuche, Y., & Le Roux,  
890 F. (2015). Populations, not clones, are the unit of *Vibrio* pathogenesis in naturally  
891 infected oysters. *The ISME Journal*, 9(7), 1523–1531.  
892 <https://doi.org/10.1038/ismej.2014.233>
- 893 Li, Y.-F., Guo, X.-P., Yang, J.-L., Liang, X., Bao, W.-Y., Shen, P.-J., Shi, Z.-Y., & Li, J.-L.  
894 (2014). Effects of bacterial biofilms on settlement of planigrades of the mussel  
895 *Mytilus coruscus*. *Aquaculture*, 433, 434–441.  
896 <https://doi.org/10.1016/j.aquaculture.2014.06.031>
- 897 Lokmer, A., & Wegner, K. M. (2015). Hemolymph microbiome of Pacific oysters in response  
898 to temperature, temperature stress and infection. *The ISME Journal*, 9(3), 670–682.  
899 <https://doi.org/10.1038/ismej.2014.160>
- 900 Lowe, D. M., & Moore, M. N. (1979). The cytology and occurrence of granulocytomas in  
901 mussels. *Marine Pollution Bulletin*, 10(5), 137–141. [https://doi.org/10.1016/0025-](https://doi.org/10.1016/0025-326X(79)90081-X)  
902 [326X\(79\)90081-X](https://doi.org/10.1016/0025-326X(79)90081-X)
- 903 Lupo, C., Amigo, A.O., Fleury, E., Robert, S., Garcia, C., Baillon, L., Béchemin, C., Canier,  
904 L., Chollet, B., Déchamps, L., Dubreuil, C., Faury, N., François, C., Godfrin, Y.,  
905 Lapègue, S., Morga, B., Travers, M.-A., Tourbiez, D., Masson, J.-C., Vérin, F.,  
906 Cordier, R., Gangnery, A., Louis, W., Mary, C., Pénot, J., Chev e, J., Dagault, F.,  
907 Jolivet, A.L., Le, D., Lebrun, L., Bellec, G., Bouget, J.-F., Cochenec-Laureau, N.,  
908 Palvadeau, H., Grizon, J., Chabirand, J.-M., P epin, J.-F., D'Amico, F., Maurer, D.,  
909 Orsoni, V., Bouchoucha, M., Roy, V.L., Pouvreau, S., Queau, I., Lamoureux, A.  
910 (2016). *Bilan 2015 du dispositif national de surveillance de la sant e des mollusques*  
911 *marins* (p. 126) [Technical Report].
- 912 Madigan, T. L., Bott, N. J., Torok, V. A., Percy, N. J., Carragher, J. F., De Barros Lopes, M.

- 913 A., & Kiermeier, A. (2014). A microbial spoilage profile of half shell Pacific oysters  
914 (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea glomerata*). *Food*  
915 *Microbiology*, 38, 219–227. <https://doi.org/10.1016/j.fm.2013.09.005>
- 916 Mallet, A.L., Carver, C.E.A., Coffen, S.S., Freeman, K.R. (1987). Mortality Variations in  
917 Natural Populations of the Blue Mussel, *Mytilus edulis*. *Can. J. Fish. Aquat. Sci.* 44,  
918 1589–1594. <https://doi.org/10.1139/f87-192>
- 919 Martenot, C., Oden, E., Travaille, E., Malas, J. P., & Houssin, M. (2010). Comparison of two  
920 real-time PCR methods for detection of ostreid herpesvirus 1 in the Pacific oyster  
921 *Crassostrea gigas*. *Journal of Virological Methods*, 170(1–2), 86–89.  
922 <https://doi.org/10.1016/j.jviromet.2010.09.003>
- 923 Metzger, M. J., Reinisch, C., Sherry, J., & Goff, S. P. (2015). Horizontal Transmission of  
924 Clonal Cancer Cells Causes Leukemia in Soft-Shell Clams. *Cell*, 161(2), 255–263.  
925 <https://doi.org/10.1016/j.cell.2015.02.042>
- 926 Metzger, M. J., Villalba, A., Carballal, M. J., Iglesias, D., Sherry, J., Reinisch, C., Muttray, A.  
927 F., Baldwin, S. A., & Goff, S. P. (2016). Widespread transmission of independent  
928 cancer lineages within multiple bivalve species. *Nature*, 534(7609), 705–709.  
929 <https://doi.org/10.1038/nature18599>
- 930 Michalek, K., Ventura, A., Sanders, T. (2016). *Mytilus* hybridisation and impact on  
931 aquaculture: A minireview. *Marine Genomics, Cells to Shells: The genomics of*  
932 *mollusc exoskeletons*, 27, 3–7. <https://doi.org/10.1016/j.margen.2016.04.008>
- 933 Moore, J. D., Elston, R. A., Drum, A. S., & Wilkinson, M. T. (1991). Alternate pathogenesis  
934 of systemic neoplasia in the bivalve mollusc *Mytilus*. *Journal of Invertebrate*  
935 *Pathology*, 58(2), 231–243. [https://doi.org/10.1016/0022-2011\(91\)90067-Z](https://doi.org/10.1016/0022-2011(91)90067-Z)
- 936 Morley, N. J. (2010). Interactive effects of infectious diseases and pollution in aquatic  
937 molluscs. *Aquatic Toxicology*, 96(1), 27–36.  
938 <https://doi.org/10.1016/j.aquatox.2009.09.017>
- 939 Moschino, V., Del Negro, P., De Vittor, C., & Da Ros, L. (2016). Biomonitoring of a polluted  
940 coastal area (Bay of Muggia, Northern Adriatic Sea): A five-year study using  
941 transplanted mussels. *Ecotoxicology and Environmental Safety*, 128, 1–10.  
942 <https://doi.org/10.1016/j.ecoenv.2016.02.006>
- 943 Najdek, M., & Sapunar, J. (1987). Total and methyl-mercury content in bivalves, *Mytilus*  
944 *galloprovincialis* Lamarck and *Ostrea edulis* Linnaeus: Relationship of biochemical  
945 composition and body size. *Bulletin of Environmental Contamination and Toxicology*,  
946 39(1), 56–62. <https://doi.org/10.1007/BF01691789>
- 947 Nasfi, H., Travers, M. A., de Lorgeril, J., Habib, C., Sannie, T., Sorieul, L., Gerard, J.,

948 Avarre, J.-C., Haffner, P., Tourbiez, D., Renault, T., Furones, D., Roque, A., Pruzzo,  
949 C., Cheslett, D., Gdoura, R., & Vallaeys, T. (2015). A European epidemiological  
950 survey of *Vibrio splendidus* clade shows unexplored diversity and massive exchange  
951 of virulence factors. *World Journal of Microbiology and Biotechnology*, 31(3), 461–  
952 475. <https://doi.org/10.1007/s11274-015-1800-y>

953 Oden, E., Burioli, E. A. V., Trancart, S., Pitel, P. H., & Houssin, M. (2016). Multilocus  
954 sequence analysis of *Vibrio splendidus* related-strains isolated from blue mussel  
955 *Mytilus* sp. during mortality events. *Aquaculture*, 464, 420–427.  
956 <https://doi.org/10.1016/j.aquaculture.2016.07.024>

957 Oden, E., Trancart, S., Pitel, P. H., & Houssin, M. (2018). Development of a Taqman<sup>®</sup> Real-  
958 Time PCR for the rapid discrimination of the *Vibrio splendidus* species among the  
959 *Splendidus* clade. *Aquaculture*, 491, 101–104.  
960 <https://doi.org/10.1016/j.aquaculture.2018.03.018>

961 Oppenheimer, C. H. and ZoBell, C. E. (1952). The growth and viability of sixty three species  
962 of marine bacteria as influenced by hydrostatic pressure. *J. Mar. Res.*, 11, 10-18

963 Peters, E.C. (1988). Recent investigations on the disseminated sarcomas of marine bivalve  
964 molluscs. *American Fisheries Society Special Publication*, 18, 74–92.

965 Petrović, S., Semenčić, L., Ozretić, B., & Ozretić, M. (2004). Seasonal variations of  
966 physiological and cellular biomarkers and their use in the biomonitoring of north  
967 adriatic coastal waters (Croatia). *Marine Pollution Bulletin*, 49(9–10), 713–720.  
968 <https://doi.org/10.1016/j.marpolbul.2004.05.004>

969 Pipe, R. K., & Coles, J. A. (1995). Environmental contaminants influencing immune function  
970 in marine bivalve molluscs. *Fish & Shellfish Immunology*, 5(8), 581–595.  
971 [https://doi.org/10.1016/S1050-4648\(95\)80043-3](https://doi.org/10.1016/S1050-4648(95)80043-3)

972 ~~Polsenaere, P., Soletchnik, P., Le Moine, O., Gohin, F., Robert, S., Pépin, J. F., Stanisière, J.-~~  
973 ~~Y., Dumas, F., Béchemin, C., & Goulletquer, P. (2017). Potential environmental~~  
974 ~~drivers of a regional blue mussel mass mortality event (winter of 2014, Breton Sound,~~  
975 ~~France). *Journal of Sea Research*, 123, 39–50.~~  
976 ~~<https://doi.org/10.1016/j.seares.2017.03.005>~~

977 Prou, J.M., Goulletquer, P. (2002). The French Mussel Industry: Present Status and  
978 Perspectives. *Bull. Aquac. Assoc. Can.* 103, 17–23.

979 Riisgård, H. U., Larsen, P. S., & Pleissner, D. (2014). Allometric equations for maximum  
980 filtration rate in blue mussels *Mytilus edulis* and importance of condition index.  
981 *Helgoland Marine Research*, 68(1), 193–198. [https://doi.org/10.1007/s10152-013-](https://doi.org/10.1007/s10152-013-0377-9)  
982 [0377-9](https://doi.org/10.1007/s10152-013-0377-9)

- 983 Robledo, J. A. F., Caceres-Martinez, J., & Figueras, A. (1994). *Mytilicola intestinalis* and  
984 *Proctoeces maculatus* in mussel (*Mytilus galloprovincialis* Lmk.) beds in Spain.  
985 *Bulletin of the European Association of Fish Pathologists*, 14(3), 89–91.
- 986 Sarmiento, A. M., DelValls, A., Nieto, J. M., Salamanca, M. J., & Caraballo, M. A. (2011).  
987 Toxicity and potential risk assessment of a river polluted by acid mine drainage in the  
988 Iberian Pyrite Belt (SW Spain). *Science of The Total Environment*, 409(22), 4763–  
989 4771. <https://doi.org/10.1016/j.scitotenv.2011.07.043>
- 990 Saulnier, D., De Decker, S., & Haffner, P. (2009). Real-time PCR assay for rapid detection  
991 and quantification of *Vibrio aestuarianus* in oyster and seawater: A useful tool for  
992 epidemiologic studies. *Journal of Microbiological Methods*, 77(2), 191–197.  
993 <https://doi.org/10.1016/j.mimet.2009.01.021>
- 994 Seed, R., & Suchanek, T. H. (1992). Population and community ecology of *Mytilus*. In:  
995 Gosling, E. (Ed.) *Developments in Aquaculture and Fisheries Science, Chap. 4. The*  
996 *mussel Mytilus: ecology, physiology, genetics and culture*, 87–169. Elsevier Science,  
997 Amsterdam.
- 998 Sheir, S. K., & Handy, R. D. (2010). Tissue Injury and Cellular Immune Responses to  
999 Cadmium Chloride Exposure in the Common Mussel *Mytilus edulis*: Modulation by  
1000 Lipopolysaccharide. *Archives of Environmental Contamination and Toxicology*, 59(4),  
1001 602–613. <https://doi.org/10.1007/s00244-010-9502-9>
- 1002 Simon, A., Bierne, N., Welch, J. J. (2018). Coadapted genomes and selection on hybrids:  
1003 Fisher's geometric model explains a variety of empirical patterns. *Evolution Letters*,  
1004 2(5): 472-498. <https://doi.org/10.1002/evl3.66>.
- 1005 Simon, A., Arbiol, C., Nielsen, E. E., Couteau, J., Sussarellu, R., Burgeot, T., Bernard, I.,  
1006 Coolen, J. W. P., Lamy, J.-B., Robert, S., Skazina, M., Strelkov, P., Queiroga, H.,  
1007 Cancio, I., Welch, J. J., Viard, F., Bierne, N. (2019) Replicated anthropogenic  
1008 hybridisations reveal parallel patterns of admixture in marine mussels. bioRxiv  
1009 590737. <https://doi.org/10.1101/590737>
- 1010 Squadrone, S., Brizio, P., Stella, C., Prearo, M., Pastorino, P., Serracca, L., Ercolini, C., &  
1011 Abete, M. C. (2016). Presence of trace metals in aquaculture marine ecosystems of the  
1012 northwestern Mediterranean Sea (Italy). *Environmental Pollution*, 215, 77–83.  
1013 <https://doi.org/10.1016/j.envpol.2016.04.096>
- 1014 Tamura, K., Stecher, G., Peterson, D., Filipinski, A., & Kumar, S. (2013). MEGA6: Molecular  
1015 Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*,  
1016 30(12), 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- 1017 Thompson, J. R., Randa, M. A., Marcelino, L. A., Tomita-Mitchell, A., Lim, E., & Polz, M.

1018 F. (2004). Diversity and Dynamics of a North Atlantic Coastal *Vibrio* Community.  
1019 *Applied and Environmental Microbiology*, 70(7), 4103–4110.  
1020 <https://doi.org/10.1128/AEM.70.7.4103-4110.2004>

1021 Travers, M.-A., Boettcher Miller, K., Roque, A., & Friedman, C. S. (2015). Bacterial diseases  
1022 in marine bivalves. *Journal of Invertebrate Pathology*, 131, 11–31.  
1023 <https://doi.org/10.1016/j.jip.2015.07.010>

1024 Venkateswaran, K., & Dohmoto, N. (2000). *Pseudoalteromonas peptidolytica* sp. nov., a  
1025 novel marine mussel-thread-degrading bacterium isolated from the Sea of Japan.  
1026 *International Journal of Systematic and Evolutionary Microbiology*, 50(2), 565–574.  
1027 <https://doi.org/10.1099/00207713-50-2-565>

1028 Viarengo, A., Canesi, L., Pertica, M., & Livingstone, D. R. (1991). Seasonal variations in the  
1029 antioxidant defence systems and lipid peroxidation of the digestive gland of mussels.  
1030 *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*,  
1031 100(1–2), 187–190. [https://doi.org/10.1016/0742-8413\(91\)90151-I](https://doi.org/10.1016/0742-8413(91)90151-I)

1032 Villalba, A., Mourelle, S. G., Lopez, M. C., Carballal, M. J., & Azevedo, C. (1993).  
1033 Marteiliasis affecting cultured mussels *Mytilus galloprovincialis* of Galicia (NW  
1034 Spain). I. Etiology, phases of the infection, and temporal and spatial variability in  
1035 prevalence. *Diseases of Aquatic Organisms*, 16, 61–72.  
1036 <https://doi.org/10.3354/dao016061>

1037 Villalba, A., Mourelle, S., Carballal, M., & López, C. (1997). Symbionts and diseases of  
1038 farmed mussels *Mytilus galloprovincialis* throughout the culture process in the Rías of  
1039 Galicia (NW Spain). *Diseases of Aquatic Organisms*, 31, 127–139.  
1040 <https://doi.org/10.3354/dao031127>

1041 Villalba, A., Carballal, M., & López, C. (2001). Disseminated neoplasia and large foci  
1042 indicating heavy haemocytic infiltration in cockles *Cerastoderma edule* from Galicia  
1043 (NW Spain). *Diseases of Aquatic Organisms*, 46, 213–216.  
1044 <https://doi.org/10.3354/dao046213>

1045 World Health Organization, 2010. Ten chemicals of major public health concern. *In: Health*  
1046 *impacts of chemicals. In: International Programme on Chemical Safety* [Online  
1047 Access]. [https://www.who.int/ipcs/assessment/public\\_health/chemicals\\_phc/en/](https://www.who.int/ipcs/assessment/public_health/chemicals_phc/en/)

1048 Yang, J.-L., Shen, P.-J., Liang, X., Li, Y.-F., Bao, W.-Y., & Li, J.-L. (2013). Larval  
1049 settlement and metamorphosis of the mussel *Mytilus coruscus* in response to  
1050 monospecific bacterial biofilms. *Biofouling: The Journal of Bioadhesion and Biofilm*  
1051 *Research*, 29(3), 247–259. <https://doi.org/10.1080/08927014.2013.764412>

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1053 **Additional file 1: Supplementary Materials and Methods**

1054 *PCR screening for bivalve mollusc known pathogens*

1055 For each mussel, a pool of minced tissues from foot, adductor muscle, gills, mantle, and  
1056 digestive gland was subjected to DNA extraction using a QIAamp DNA minikit<sup>®</sup> (Qiagen,  
1057 Courtaboeuf, France) following the manufacturer's protocol for blood or body fluids, except  
1058 for elution performed in 60 µL Qiagen elution buffer AE. The quality of the extracted DNA  
1059 was checked with NanoDrop<sup>™</sup> 2000c spectrophotometer (ThermoFisher Scientific<sup>™</sup>,  
1060 Waltham, MA USA). Within the same batch, the individual extracts were pooled in groups of  
1061 5 to have 4 replicates per batch for each PCR done. The known bivalve pathogens  
1062 investigated by TaqMan<sup>®</sup> real-time PCR and classical PCR were: *Bonamia* spp.,  
1063 *Haplosporidium nelsoni*, *Marteilia* sp., *Mikrocytos mackini*, *Nocardia crassostreae*, Ostreid  
1064 Herpesvirus type 1 (OsHV-1), *V. aestuarianus*, *V. tubiashii*, *V. harveyi*, and *V. splendidus* (the  
1065 true species *V. splendidus* of the Splendidus clade named here Splendidus cluster) (Table S1).  
1066 Amplification reactions were performed in a total volume of 25 µL using a SmartCycler<sup>®</sup>  
1067 (Cepheid, USA). Each reaction contained 12,5 µL of a Premix Ex Taq<sup>®</sup> 2 X Takara<sup>®</sup> (Lonza,  
1068 Verviers, Belgium), 9 µL of purified water, 2 µL of DNA sample (replaced with 2 µL of  
1069 purified water in the negative control) and 0.5 µL of each primer (20 µM) and probe (10 µM)  
1070 or SYBR Green (for *N. crassostreae*). Each assay included negative and positive control  
1071 reactions. The thermal cycle profile consisted of 95 °C for 10 s followed by 40 cycles of 95  
1072 °C for 5 s and 60° for 30 sec (or 62 °C for *V. splendidus* or 64 °C for *V. harveyi*). Beforehand,  
1073 for each real-time PCR developed for this study, the inclusivity and exclusivity were tested.  
1074 Moreover, in order to exclude false-positive results, when a signal was obtained, the amplicon  
1075 was sent to Eurofins MWG Operon (Ebersberg, Germany) to be purified and subsequently  
1076 sequenced; only then, after using the NCBI BLAST, confirmation of the targeted pathogen  
1077 could be made.

Table S1. Target genes, primers (forward (Fw) and reverse (Rv)) and probe (Pr) used for detection of known bivalve molluscs pathogens.

Name of pathogens	Target gene	Oligo-nucleotides sequences (5'-3')	References
<i>Bonamia</i> spp.	28S rRNA	Fw TCCCTGCCCTTTGTACACA Rv CTCTTATCCACCTAATTCACCTCAG Pr TxR-CGCCCGTCGCTTCTACCGATT-BHQ2	Present paper
<i>Haplosporidium nelsoni</i>	SrRNA	Fw CACGCGCGCTACAATGT Rv CGAGATTACCCGGCCTTCT Pr FAM-CACGCAACGAGTTCAACCTTGCC-BHQ1	Present paper
<i>Marteilia</i> sp.	ITS1	Fw CACTACTCTTCGCTTTTCGAT Rv GACTACCCGTGCCGAACA Pr Cy3-TCGCAAACAGGAAGCGGCTCTC-BHQ1	Present paper
<i>Mikrocytos mackini</i>	28S rRNA	Fw GGTGGCCGAATGACGTAGT Rv GCCTATGACAGCACGAAGCA Pr Cy5-CCGCTTCGGCGTGCAGTCTC-BHQ2	Present paper
<i>Nocardia crassostreae</i>	16S-23S rNA ITS	Fw CCTCGATACCGCCGAAGAA Rv CAACACACCCGCATCAA	Carrasco <i>et al.</i> (2013)
OsHV-1	B region	Fw GTCGCATCTTTGGATTTAACAA Rv ACTGGGATCCGACTGACAAC Pr TxR-TGCCCTGTCATCTTGAGGTATAGACAATC-BHQ2	Martenot <i>et al.</i> (2010)
<i>V. aestuarianus</i>	dnaJ	Fw GTATGAAATTTTAACTGACCCACAA Rv CAATTTCTTTCGAACAACCAC Pr TxR-TGGTAGCGCAGACTTCGGCGAC-BHQ2	Saulnier <i>et al.</i> (2009)
<i>V. tubiashii</i>	vtpA	Fw GGTACGGACTATCCGGGATT Rv TTCACCGCTGAGTTGTTTCAT Pr Cy3-ATCGTCGATAAATCAGGCACAACCTGT-BHQ1	Present paper
	vth	Fw CGGTTGATATTCGCGTCAA Rv GTGTGAAACCCTGCGAAGTA Pr Cy5-TATCACAGATGCGCTCGGTTTCAGTC-BHQ2	Present paper
<i>V. harveyi</i>	16S rRNA	Fw CGAGCGGAAACGAGTTATCTG Rv CTCACCAACTAGCTAATCCACCTA Pr TxR-CCGCATAATACCTACGGGTCAAAGAGGG-BHQ2	Present paper
<i>V. splendidus</i> ( <i>Splendidus</i> cluster)	toxR	Fw AGCAGCGGCTGAAATTGCA Rv GGCCGCAGTTGGTGTGTT Pr FAM-CAATGACTGAAGCTGTTCGAGCCC-BHQ1	Oden <i>et al.</i> (2018)