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1 **Impact of epizootics on mussel farms: Insights into microbiota composition of *Mytilus***
2 **species**

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14 **Abstract**

15 Outbreaks of marine mussel mortality on French farms could have different aetiologies. One of
16 them implies *Vibrio splendidus* strains. Beyond the involvement of this pathogen, there is
17 considerable evidence that diseases often result from interactions between several microbes and
18 the host. In this study, we explored the bacterial communities associated with mussel species
19 and the surrounding water collected from a mussel farm affected by mortalities. The microbiota
20 of *Mytilus edulis*, *Mytilus galloprovincialis* and their hybrids displayed an abnormal abundance
21 of Proteobacteria, in particular the genera *Vibrio*, *Cobetia* and *Arcobacter*. Despite the
22 dysbiosis, the Mediterranean mussel showed a different microbiota profile with a higher
23 richness and presence of the phylum Bacteroidetes. Bipartite network analyses at the level of
24 bacteria families confirmed this finding and showed that the microbiomes of *M. edulis* and the
25 hybrids tended to cluster together. In addition, injection of mussels with the virulent *V.*
26 *splendidus* induced less mortality rate in *M. galloprovincialis* compared to the other *Mytilus* sp.
27 suggesting a better resistance of the Mediterranean mussel to infection.

28 Our findings point to a probable aetiology of pathobiome-mediated disease in mussels. To fully
29 understand this phenomenon, more knowledge is needed on the roles of pathobiotic systems
30 and their development during disease establishment.

31 **Keywords:** Pathobiome, mussel mortalities, opportunistic bacteria, core microbiome

32 **1. Introduction**

33 Over the last two decades, European mussel production has been declining. Among the putative
34 causes of this trend, the spread of diseases, algal blooms, lack of spat, predation and low
35 profitability have been mentioned (Avdelas et al., 2021). Indeed, since 2014, mass mortality
36 events have been recorded in blue mussel stocks in France (Bechemin et al., 2014) and since
37 2016 in the Netherlands (Capelle et al., 2021). While no cause could explain this phenomena in
38 the Netherlands (Capelle et al., 2021), the involvement of pathogens in mortalities has been
39 demonstrated in French mussel farms (Ben Cheikh et al., 2017, 2016; Benabdelmouna et al.,
40 2018; Oden et al., 2016).

41 As filter-feeding organisms, marine mussels are constantly exposed to a variety of
42 microorganisms including pathogenic bacteria that can threaten their lives. In addition to
43 external microbes, like all macroorganisms, mussels interact with the microorganisms they
44 harbour, i.e. their microbiota. This dialogue between the host and its microbiota can help
45 maintain their respective integrity, as shown e.g. in leeches (Kikuchi and Graf, 2007; Tasiemski
46 et al., 2015). In fact, advances in analytical techniques over the last few decades have favoured
47 the expansion of studies on microbial communities, particularly in the human model,
48 highlighting their importance for their host (Amato, 2016; Bäckhed et al., 2005; Martin et al.,
49 2009; Mushegian and Ebert, 2016). Considered the “last organ” in humans (Baquero and
50 Nombela, 2012), a large body of evidence has revealed the beneficial contribution of the
51 microbiota to basic biological processes such as digestive, metabolic and immune functions
52 (Callens et al., 2016; Sirisinha, 2016; Wang et al., 2017) and its involvement in the eco-
53 evolutionary dynamics of their host (Amato, 2016; Kohl et al., 2018; Macke et al., 2017;
54 Moeller et al., 2016; Murall et al., 2017). However, like other ecosystems, the composition of
55 the microbiota can be modulated by several factors such as antibiotics and pathogens leading
56 to an altered community structure or dysbiosis (Clark et al., 2015; Costello et al., 2012; Lamont

57 and Hajishengallis, 2015; Martin et al., 2009). In case of dysbiosis, the mutual harmony
58 between bacteria is disrupted and the healthy balanced microbiota turns into a disease-inducing
59 pathobiota (Pitlik and Koren, 2017; Tunland, 2018; Wang et al., 2017).

60 The large number of findings in the human model has sparked research interest in other
61 organisms including bivalves. Numerous studies have investigated microbiota structure in
62 oysters, mussels and to a lesser extent clams, under the influence of factors such as host
63 genotype, environment and pathogens (for review see Paillard et al., 2022). The structure of the
64 microbiota can be divided into at least two types of communities, namely a stable community
65 of resident bacteria within a host population (“the core microbiome”) and a variable microbial
66 community that depends on the surrounding environment (Santibáñez et al., 2022). Data on the
67 role of the core microbiome are limited, although some authors suggest a contribution to
68 essential host biological functions (Lemanceau et al., 2017). Furthermore, the balance between
69 the core and variable microbiome is still unclear, especially as the dynamics of host-associated
70 communities are directly affected by abiotic and biotic factors. For example, elevated water
71 temperature appears to be a major determinant and strongly influences the composition of the
72 microbiota in mussels and oysters by also favouring pathogens such as *Vibrio* sp. (Li et al.,
73 2019, 2018; Lokmer and Wegner, 2015). In addition, water pollution by nanoparticles,
74 nanoplastics or chemicals can affect the diversity of the microbiota as has been demonstrated
75 in *Mytilus galloprovincialis* (Auguste et al., 2020, 2019; Balbi et al., 2020). Besides abiotic
76 factors, microbiota imbalance has been linked to diseases (King et al., 2019). Indeed, infection
77 of *Crassostrea gigas* by OsHV-1 virus induced dysbiosis with subsequent bacteraemia by
78 opportunistic bacteria (Lorgeril et al., 2018). Similarly, field studies on oysters affected by
79 mortalities have shown signs of microbiota disruption (Lasa et al., 2019).

80 For mussels, only a few studies have explored microbiota dynamics in interaction with
81 pathogens. Li et al. (2019) reported the reduction in microbial diversity of *M. coruscus*

82 haemolymph after exposure to *Vibrio cyclitrophicus*. Likewise, we recently demonstrated that
83 experimental infection with *V. splendidus* reduces the diversity of the *M. edulis* microbiota and
84 favours pathobiontic bacteria (Ben Cheikh and Travers, 2022). However, the response of the
85 mussel microbiota during disease outbreaks remains unknown.

86 The aim of this study is to provide new insights into the microbiota of mussels experiencing
87 mortality outbreaks on French farms. For this purpose, the bacterial communities associated
88 with *M. edulis*, *M. galloprovincialis* and their hybrids, grown in the same environment, and the
89 surrounding water were analysed. The differences between the microbial communities found in
90 the different species were assessed using ecological network methods to cluster microbiota and
91 link the obtained classification to the type of sampled mussels. The susceptibility of the
92 different types of mussels to infection was then explored by experimental infection with *V.*
93 *splendidus* strains.

94 **2. Material and methods**

95 **2.1. Mussel collection**

96 45 farmed adults of *Mytilus* sp. (*M. edulis*, *M. galloprovincialis* and hybrid *M. edulis* x
97 *galloprovincialis*, 15 individuals from each taxon, size between 4 and 5 cm) were collected
98 from a mussel farm affected by massive mortalities (Bay of Lannion, France 48°44 44.8 N,
99 3°35 24.9 W) in September 2018 (mean temperature = 17°C). The animals were immediately
100 transported to the laboratory and randomly dispatched in different temperature-controlled
101 (16°C) aerated water tanks filled with seawater sampled from the corresponding site (salinity =
102 35 PSU). Mussels were taken at random from two different tanks for microbiota analysis or for
103 experimental infection. Seawater from the same site was also sampled for microbiota analyses.

104 **2.2. Microbiota analysis**

105 After cleaning, mussels were removed from their shells, flash frozen in liquid nitrogen and
106 stored at -80°C (15 individuals/species). Serial filtrations of 5L of seawater were performed
107 using 8 µm and 5 µm porosity (Whatman™ Nucleopore™ Polycarbonate membrane filter,
108 47 mm diameter, UK). The resulting filtered seawater was centrifuged for 15 min at 11000 g.
109 The membranes and bacterial pellets were stored at -80°C.

110 DNA extraction, amplicon library construction and Illumina MiSeq sequencing were performed
111 by Biofidal (Vaulx-en-Velin, France). DNA was extracted from frozen mussels ground in liquid
112 nitrogen and from bacteria pellet using Mag Bind® Universal Pathogen DNA Kit (Omega BIO
113 TEK, Georgia, USA) and from the different filters using Quick DNA/RNA MagBead kit (Zymo
114 Research, California, USA) according to the manufacturer's protocol. DNA concentration and
115 quality were checked with the QuantiFluor® dsDNA System (Promega, France). The 16S
116 rRNA gene of bacterial communities was amplified using the 5X HOT BIOAmp ®
117 BlendMaster Mix (Biofidal) and the 341F: CCTACGGGNGGCWGCAG (17 pb) and 805R:
118 GACTACHVGGGTATCTAATCC (21 pb) primers (designed by Biofidal) targeting the
119 variable V3-V4 loops (Klindworth et al., 2013). After purification of the PCR products by
120 SPRIselect reagent kit (Beckman Coulter), a second amplification was performed using index
121 adapter oligos (P5/P7). The purified PCR products were normalized to obtain an equimolar
122 library and subsequently pooled. Negative controls and ZymoBIOMICS microbial community
123 standards were included during the library preparation (Supplemental Figure S1). Amplicons
124 were sequenced on the MiSeq platform with V3 reagents producing 2 X 300 bp paired-end
125 reads.

126 After demultiplexing, primer sequence adapters were trimmed from the reads using cutadapt
127 software version 1.12 (Martin, 2011). Paired-end sequence reads were collapsed into so-called
128 pseudoreads using sequence overlap with USEARCH version 9.2 (Edgar, 2010). After chimera

129 and singletons removing, filtered pseudoreads were aligned to the RDP database version 11.5
130 (Cole et al., 2014) with Snap-aligner version 1.0 beta.23 (Zaharia et al., 2011) to identify the
131 taxonomy. Finally, USEARCH was used to create the OTU-table and to calculate alpha
132 diversity. Principal coordinate analyses (PCoA) were performed via xlstat using the Bray–
133 Curtis distance matrix. All FastQ files were deposited in SRA-NCBI under the project accession
134 number PRJNA923705.

135 **2.3. Host-microbiota network**

136 To analyse the structure of the host-microbiota association dataset, we used methods borrowed
137 from the analysis of bipartite ecological networks. First, we aggregated data on microbial taxa
138 at the family level and transformed the microbial family abundance table into presence/absence
139 data (i.e., all abundances > 0 were set to 1). We then modelled the obtained bipartite network
140 of hosts by microbial families using a latent block model (LBM) (Govaert and Nadif, 2008;
141 Leger et al., 2015). The clustering obtained with LBMs is indeed more useful than the one
142 obtained with modularity-search algorithms (Newman, 2006; Traag et al., 2019) as blocks can
143 identify densely connected as well as sparsely connected regions of the network. We tested the
144 correlation between the obtained clustering of hosts and their classification among species using
145 a method described in Massol et al. (2021): we computed the normalised mutual information
146 (NMI) index between the two classifications of the hosts (by host type and by LBM block); we
147 tested the significance of NMI using randomizations of the bipartite network through the
148 configuration model (Strona et al., 2014).

149 **2.4. Experimental infection**

150 Two strains of *V. splendidus*, the virulent 10/068 1T1 and the non-virulent 12/056 M24 T1 were
151 used for experimental infection by injection as described in previous studies (Ben Cheikh et al.,
152 2017, 2016). Briefly, bacteria were grown overnight in Luria Bertani NaCl 20 g.L⁻¹ at 22°C

153 with constant agitation (80 rpm) and resuspended in filtered sterile seawater (FSSW, OD_{600nm}
154 = 1, 2.10^8 CFU mL^{-1}) after double washing (FSWW) /centrifugation (1200 g for 10 min).
155 Anesthetized mussels (2–3 h in magnesium chloride solution at 50 $g.L^{-1}$, 1/4: v/v
156 seawater/freshwater) were intramuscularly injected with 100 μL of bacterial suspension or
157 FSSW for negative controls. The animals were transferred to tanks (3 replicate tanks, 10
158 mussels per tank) filled with 2 L of UV-treated and filtered seawater supplemented with 50 mL
159 of phytoplankton (*Isochrysis galbana*) and maintained under static conditions at 16°C with
160 aeration. Mortality was monitored over 8 days with removing of newly dead mussels from the
161 tanks.

162 **2.5. Statistical analyses**

163 SigmaPlot 12 (Systat Software Inc., Chicago, IL) and R version 4.0.5 (R Core Team, 2021)
164 were used for statistical analysis. A comparison of OTUs between mussel types and/or seawater
165 was performed. Values were tested for normality (Shapiro-Wilk test) then a one-way ANOVA
166 was performed. Pairwise comparisons between groups were assessed by post-hoc analyses
167 (with Holm-Sidak correction). Statistical significance was accepted for $p < 0.05$.

168 **3. Results**

169 **3.1. Microbial structure in mussels and seawater**

170 The 48 samples (15 *M. edulis*, 15 *M. galloprovincialis*, 15 hybrid and 3 seawater samples)
171 sequenced using the 16S rRNA gene generated 4 842 875 raw reads ranging from 70 302 to
172 126 377 reads per sample with an average of 100 893 reads (Supplemental Table S1). After
173 Chimera filtering, unique pseudoreads averaged 38 597 reads.

174 The clustering of reads in OTUs revealed a higher richness in the Mediterranean mussel *M.*
175 *galloprovincialis* (significant difference with *M. edulis*, ANOVA $p < 0.05$) and in seawater with
176 a mean absolute abundance of $80\,486 \pm 3\,214$ OTUs and $80\,367 \pm 808$ OTUs respectively. The

177 blue mussel *M. edulis* and the hybrids had a mean absolute abundance of $68\,068 \pm 2\,322$ and
178 $70\,637 \pm 2\,783$ OTUs respectively (Figure 1 A). Alpha diversity metrics were higher in *M.*
179 *galloprovincialis* compared to the other two mussel species and seawater (non-significant data,
180 supplemental Figure S2A). Beta diversity analysis revealed dissimilarities between mussels and
181 seawater (Supplemental Figure S2B). The compositional structure at the phylum level indicated
182 a similarity between *M. edulis* and the hybrids (Figure 1 B) with a predominance of
183 Proteobacteria (relative abundance r.a. of 92.3% to 99.4% in *M. edulis* and 89.9% to 99.9% in
184 hybrids) and the presence of Bacteroidetes (r.a. from 0.3% to 5.5% in *M. edulis* and from 0 to
185 5.4% in hybrids). Other rare phyla with an abundance > 1 were detected in some hybrids, e.g.,
186 Firmicutes (replicates 2, 6 and 7) and Tenericutes (replicates 1 and 13). Mediterranean mussels
187 harboured a lower proportion of Proteobacteria (r.a. from 47.3% to 99.6%) and a higher
188 proportion of Bacteroidetes (r.a. from 0 to 23.5%). OTUs belonging to Tenericutes,
189 Verrucomicrobia, Fusobacteria and Cyanobacteria were also identified in some mussels with
190 r.a. > 1 . The composition of the surrounding seawater was similar to that of the mussels with a
191 notable r.a. of Bacteroidetes (mean $21.1\% \pm 6.4\%$) and the predominance of Proteobacteria
192 (mean $77.4\% \pm 6.9\%$).

193 A comparative analysis of the most abundant families (Top 30) and genera revealed a more
194 specific layout for each group although a similar bacterial community was detected in most
195 samples (Figure 2, Supplemental Table S2). Overall, Vibrionaceae, Halomonadaceae,
196 Pseudoalteromonadaceae and Campylobacteraceae were the dominant families in the blue
197 mussel and hybrids (mean r.a. 5-35%) with notably the genera *Vibrio*, *Cobetia*,
198 *Pseudoalteromonas* and *Arcobacter* but also unclassified Epsilonproteobacteria. Similarly, the
199 Mediterranean mussel harboured Vibrionaceae (*Vibrio*), Pseudoalteromonadaceae
200 (*Pseudoalteromonas*) and unclassified Epsilonproteobacteria but also a higher proportion of
201 Campylobacteraceae (*Arcobacter*) and Flavobacteriaceae (especially *Tenacibaculum* and

202 *Flavobacterium*) (mean r.a. 18.2% and 6.8% respectively). In contrast, in addition to
203 unclassified Gammaproteobacteria, the prevailing taxa (r.a. up to 5%) in seawater samples were
204 the families Rhodobacteraceae, Oceanospirillaceae, Pseudoalteromonadaceae,
205 Crocinitomicaceae and Flavobacteriaceae and the genera *Sulfitobacter*, *Oceanospirillum*,
206 *Pseudoalteromonas*. The majority of *Vibrio* identified in mussels have been affiliated to the *V.*
207 *Splendidus* group (Supplemental Table S3).

208 **3.2. Association between microbial family structure and mussel taxa**

209 The LBM inferred 5 clusters of hosts and 4 clusters of bacterial families (Figure 3A).
210 Assignment of host individuals to clusters resulted in probabilities of membership at least as
211 high as 88.3%; by contrast, a few bacterial families were only assigned to a particular cluster
212 with a probability of 50.8% (Supplemental Figure S3). The first cluster of hosts consisted in 2
213 samples, one of *M. galloprovincialis* and one hybrid, which harboured high numbers of
214 bacterial families (223 families on average). The second cluster of hosts consisted in 20 samples
215 (1 hybrid, 7 *M. edulis* and 12 *M. galloprovincialis*) with an average of 141.9 bacterial families
216 per sample. The third cluster included only 4 samples (one replicate of *M. galloprovincialis*
217 together with the three seawater samples) with an average of 125.5 families per sample. The
218 fourth cluster of hosts consisted in 16 samples (7 *M. edulis*, 1 *M. galloprovincialis*, 8 hybrids)
219 with an average of 109 families per sample. The fifth cluster of hosts consisted in 6 samples (1
220 *M. edulis*, 5 hybrids) with an average of 69 families per sample. The four clusters of bacterial
221 families harboured 56, 57, 89 and 176 families, respectively, and were found on average in
222 45.46, 30.44, 13.44 and 2.63 host samples respectively. Probabilities of host-bacterial family
223 interactions can be found in Supplemental Table S4.

224 The classification of the hosts (48 samples) according to their LBM cluster was significantly
225 congruent with their classification based on sample type (mussel taxon or seawater sample, i.e.,
226 4 groups) according to the NMI (NMI = 0.381, p value < 0.0001 when tested against 10,000

227 randomizations of the bipartite network). Indeed, a graphical representation of the two
228 classifications evince that *M. galloprovincialis* projects almost completely onto LBM cluster 2
229 while LBM clusters 4 and 5 project mostly onto *M. edulis* and hybrids (Figure 3B). Seawater
230 samples coincide almost perfectly with LBM cluster 3.

231 **3.3. Susceptibility of mussels to pathogens**

232 To assess the susceptibility of mussel populations to bacterial infection, *M. edulis*, *M.*
233 *galloprovincialis* and hybrids were injected with two strains of *V. splendidus* of contrasting
234 virulence. Exposure of mussels to avirulent strain 12/056 M24 T1 induced limited mortalities
235 in all mussel groups, ranging from $3.3\% \pm 3.3\%$ in hybrids to $13.3\% \pm 3.3\%$ in *M. edulis* at day
236 8 (Figure 4). In contrast, injection of the virulent strain 10/068 1T1 induced higher mortalities
237 with different rates in the mussel species. The first mortalities appeared 24h post-injection and
238 increased progressively until stabilization on day 8. Hybrids and *M. edulis* had different patterns
239 at the beginning of the infection ($16.6\% \pm 12\%$ for *M. edulis* and $46.6\% \pm 6.6\%$ for hybrids on
240 day 1) but reached 76.6% of mortality at the end of the experiment while *M. galloprovincialis*
241 had lower mortalities ($23.3\% \pm 3.3\%$ on day 1 and $46.6\% \pm 12\%$ on day 8). No or negligible
242 mortality was observed in controls.

243 **4. Discussion**

244 Since 2014, mass mortalities have been reported in French mussel farms. While *M. edulis*, *M.*
245 *galloprovincialis* and their hybrids represent the main farmed species, the epizootics seem to
246 mostly affect the blue mussel (Bechemin et al., 2014). In general, investigations of mussel
247 disease have so far been limited to the detection and isolation of known pathogens from
248 moribund animals such as *V. splendidus* (Oden et al., 2016), combined with experimental
249 infection in the laboratory (Ben Cheikh et al., 2017, 2016). In this study, we provide an
250 overview of the bacterial communities associated with three mussel species and the surrounding
251 water sampled from a farm affected by mortality epidemics.

252 **4.1. The microbiota of the three *Mytilus* sp. is dysbiotic**

253 According to previous studies, the microbiota of healthy marine bivalves, and mussels in
254 particular is characterized by the presence of different phyla with a predominance of
255 Proteobacteria but also other bacteria belonging to Cyanobacteria, Firmicutes, Planctomycetes,
256 Bacteroidetes, Actinobacteria, Verrucomicrobia, Tenericutes and Chloroflexi (Ben Cheikh and
257 Travers, 2022; Musella et al., 2020). Here, the analysis of whole animal microbiota showed a
258 severe dysbiosis marked by an abnormal dominance of Proteobacteria (Fig. 1, mean r.a. ranging
259 from 86.5% to 96.1%). Microbiota imbalance has been linked to mortality events and infectious
260 diseases in bivalves. Field analysis of the Pacific oyster *C. gigas* showed that animals
261 experiencing mortality outbreaks at different life stages displayed signs of dysbiosis resulting
262 in a change in the diversity and composition of the microbiota (Lasa et al., 2019). Similarly, a
263 study on Unionida freshwater mussel during mass mortality events revealed bacterial invasion
264 and shifts in the bacterial microbiome (Richard et al., 2021). For marine mussels, there are no
265 data available to our knowledge on animal microbiota during mortality events. However, we
266 have recently reported a disruption of the blue mussel microbiota during experimental infection
267 with *V. splendidus* and demonstrated the implication of some opportunistic pathobiontic
268 bacteria in the infection (Ben Cheikh and Travers, 2022). Thus, even if the causal origin of
269 mussel mortalities in France still not clear, we can establish a link with pathogenic bacteria,
270 especially as they are detected in *Mytilus* tissues. Our analysis highlighted the genera *Vibrio*,
271 *Cobetia* and *Arcobacter* as the predominant bacteria in mussel species (Fig. 2). The abundance
272 of *Vibrio* sp. in the microbiota of mussels is not surprising as these genera are naturally
273 occurring in marine environments and certain strains are often associated with outbreaks of
274 mortality marine bivalves (Travers et al., 2015). In our study, we mainly detected strains
275 belonging to the *V. Splendidus* clade which includes pathogens of bivalves such as *V.*
276 *splendidus*, *V. tasmaniensis* and *V. crassostreae* (Ben Cheikh et al., 2016; Bruto et al., 2017;

277 Duperthuy et al., 2011) but also less studied strains showing virulence for some marine
278 organisms (e.g. *V. toranzoniae* and *V. kanaloae*) and environmental species without pathogenic
279 potential (e.g. *V. gigantis* and *V. atlanticus*) (Lasa et al., 2017; Romalde et al., 2014). In
280 accordance with our findings, different studies have reported the presence of *V. splendidus*-
281 related strains in different French mussel farms affected by mortalities (Bechemin et al., 2014;
282 Charles et al., 2020a; Oden et al., 2016). However, these studies are restrictive as they are based
283 on the isolation of culturable bacteria of interest and are therefore not representative of the
284 animal's microflora. In addition to *V. splendidus* strains, *Francisella halioticida* has been
285 recently described as potentially involved in blue mussel mortality (Cano et al., 2022; Charles
286 et al., 2020b). The implication *F. halioticida* in mussel disease is not confirmed in our study
287 since this strain was detected at very low proportions in some individuals of blue mussels and
288 hybrids (data not shown). In contrast, metagenomic analyses showed the prevalence of bacteria
289 of the genus *Cobetia* and *Arcobacter*. While knowledge of *Cobetia* strains is limited, several
290 reports described the dominance of *Arcobacter* sp. in oysters and mussels in a context of disease
291 suggesting them as opportunistic bacteria (Ben Cheikh and Travers, 2022; Li et al., 2019;
292 Lokmer and Wegner, 2015; Lorgeril et al., 2018). Nevertheless, the potential role of these
293 bacteria needs further investigation, as some species (e.g. *A. cryaerophilus*, *A. ellisii*, *A.*
294 *nitrofigilis*) have virulence genes and the ability to colonise and enter human cells (Ferreira et
295 al., 2016; Levican et al., 2013).

296 **4.2. Microbiota of *M. galloprovincialis* differs from *M. edulis* and hybrids**

297 The analysis of microbiomes using a latent block model identified four clusters of bacteria
298 families and five clusters of host individuals (Fig. 3A). Since this clustering proved to be
299 congruent with the one obtained from host types, with a significantly high NMI, this suggests
300 that microbiota are different between mussel species. Indeed, host clusters 4 and 5 belong
301 almost exclusively to *M. edulis* and hybrids, while *M. galloprovincialis* individuals are almost

302 all found in host cluster 2. The differences in bacteria family richness between clusters thus
303 confirm that *M. galloprovincialis* is associated to a richer microbiome than both *M. edulis* and
304 hybrids. The microbial cluster 3 is more associated with host cluster 2 than with host clusters 4
305 and 5 (probabilities of interactions: 36.6% vs. 18.1% and 8.7%; Supp. Table S4). Therefore, it
306 probably contains some of the bacterial families peculiar to *M. galloprovincialis*. Despite these
307 differences, the latent block model also uncovered a potential list of bacterial families that could
308 constitute the ‘core microbiome’ of all sampled mussels, i.e., microbial cluster 1. This group of
309 56 bacterial families comprises quite common families such as Aeromonadaceae,
310 Enterobacteriaceae, Pseudomonadaceae, Rickettsiaceae and Vibrionaceae (Supp. Table S5). In
311 particular, this cluster contains the three families comprising the three genera described in Fig.
312 2 (Halomonadaceae, Campylobacteraceae and Vibrionaceae).

313 The shared core microbiota among the three mussel species confirms that the surrounding
314 environment plays a major role in shaping the composition of the microbiome. In agreement
315 with our findings, previous reports have shown that the microbiota of co-cultivated oysters and
316 mussels displayed a high degree of similarity (Pierce and Ward, 2019; Vezzulli et al., 2018).
317 However, host genetics also appears to be a driving factor in the mussel microbiota given the
318 dissimilarity between the microbial communities associated with *M. galloprovincialis* and the
319 other two species. The specific microbiome of the Mediterranean mussel may confer fitness
320 advantages to the host including resistance and survival from disease as demonstrated in other
321 organisms (Kueneman et al., 2016; Madison et al., 2022; Zackular et al., 2013). Compared to
322 *M. edulis* and hybrids, *M. galloprovincialis* showed a lower abundance of *Cobetia* and
323 *Arcobacter* species. In addition, despite the high prevalence of *V. Splendidus* strains, the
324 Mediterranean mussel survived experimental infection with the virulent *V. splendidus* 10/068
325 1T1 better (Fig. 4). Resistance of *M. galloprovincialis* to infectious diseases has been previously
326 reported. Fuentes et al. (2002) described higher warm season mortality of *M. galloprovincialis*

327 x *M. edulis* hybrids than *M. galloprovincialis* individuals. Similarly, Benabdelmouna et al.
328 (2018) suggested a higher susceptibility in *M. edulis* relative to *M. galloprovincialis* at the spat
329 stage, which is consistent with field mortalities. Thus, the microbiome of *M. galloprovincialis*
330 may be related to the efficiency of defence mechanisms and resistance to infectious diseases.
331 Characterization of host-microbiota dynamics at the spatial and temporal scales will be crucial
332 to understanding the roles of the microbiome in maintaining host homeostasis.

333 **5. Conclusions**

334 In this study, we provide for the first time an overview of the microbiota of *M. edulis*, *M.*
335 *galloprovincialis* and their hybrids in the context of mortalities in French mussel farm. We
336 reported dysbiosis of the microbiota in the three mussel species and a difference between
337 bacterial communities in seawater and mussel tissues but also between mussel species. Among
338 the pathobiontic bacteria detected, members of *Vibrio*, *Cobetia* and *Arcobacter* genera were the
339 most abundant suggesting their involvement in mussel disease. In addition, *M. galloprovincialis*
340 showed a different microbiota profile and was also more resistant to infection by *V. splendidus*.
341 Thus, we can speculate on the link between the microbiota of Mediterranean mussels and their
342 resistance to infectious disease. Further research is needed to understand the nature of the
343 interactions of mussel microbiota, including the pathobiome, with their host, which may
344 ultimately help to manage and monitor host health to prevent mortalities in mussel farms.

345 **CRedit authorship contribution statement**

346 **Yosra Ben Cheikh:** Conceptualization, Methodology, Validation, Formal analysis,
347 Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, supervision.

348 **François Massol:** Methodology, Formal analysis, Writing - Original Draft, Writing - Review
349 & Editing, Visualization. **Nathalie Giusti-Petrucciani:** Investigation. **Marie-Agnès Travers:**
350 Conceptualization, Writing - Review & Editing.

351 **Data availability statement**

352 The data that support the findings of this study are available from the corresponding author
353 upon reasonable request.

354 **Declaration of competing interest**

355 The authors declare no conflicts of interest.

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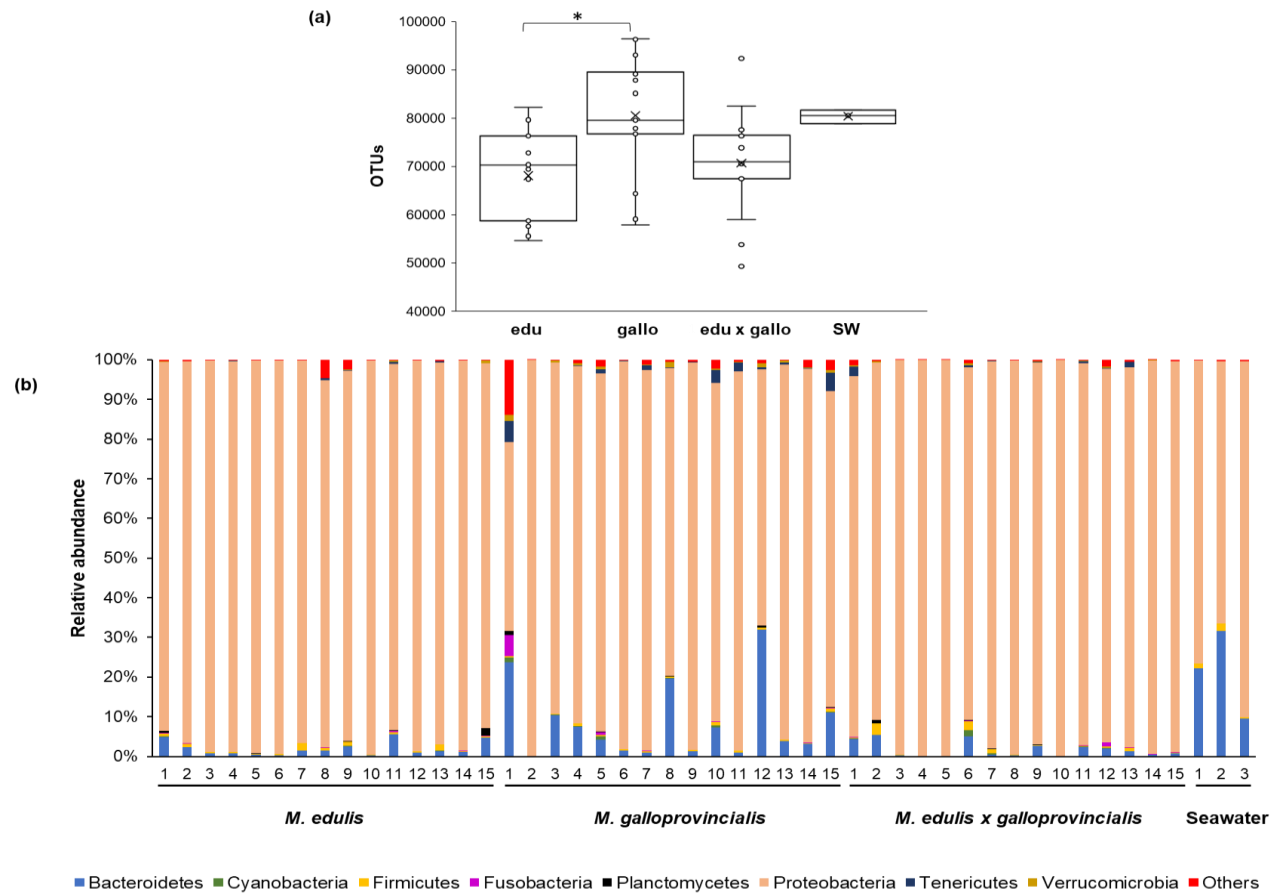
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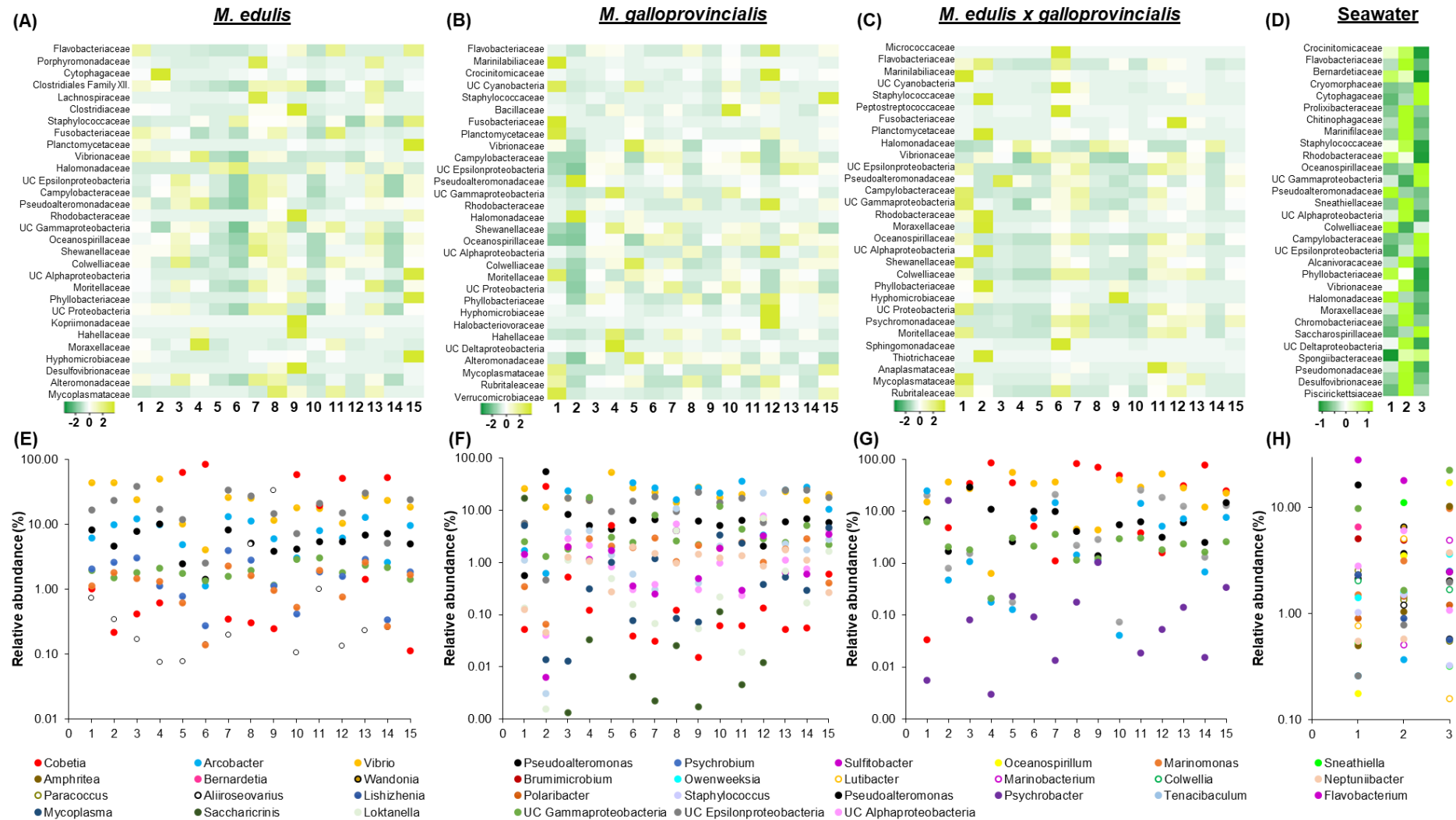
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595 **Figure 1. Total OTUs and the relative abundance of bacterial phyla in mussels and seawater.** (A) total OTUs presented by box-and-whisker
 596 plots showing high, low, and median values, with lower and upper edges of each box denoting first and third quartiles, respectively and the mean
 597 with a cross. Edu = *M. edulis*, gallo = *M. galloprovincialis*, edu x gallo = *M. edulis* x *galloprovincialis* and SW = seawater. * indicates significant
 598 difference $p < 0.05$ (ANOVA, Holm-Sidak test); (B) relative abundance of bacterial communities at the phylum level. Replicates are labelled with
 599 the numbers 1 to 15 for animals and 1 to 3 for seawater.



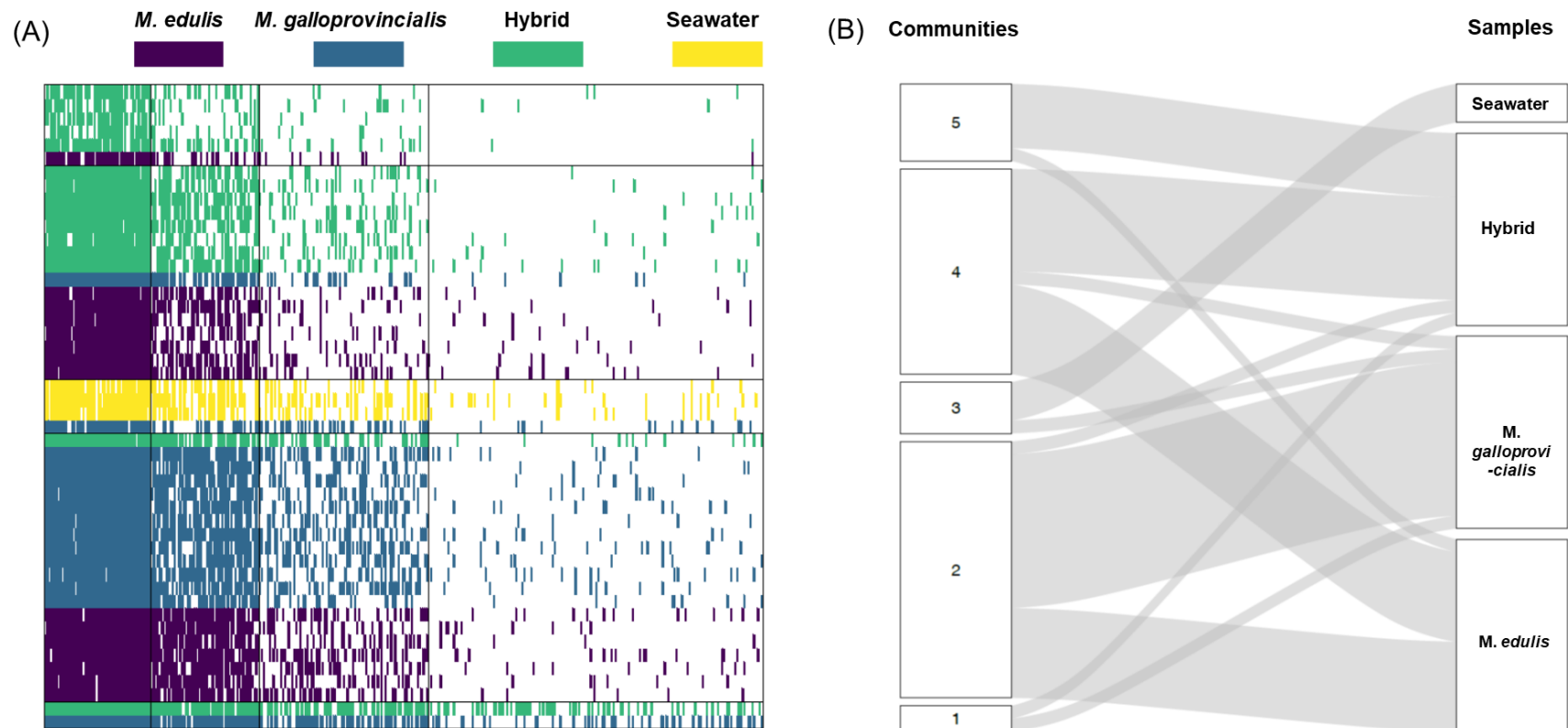
601 **Figure 2. The most abundant bacterial family and genera in *Mytilus* sp. and seawater.** (A-D) heatmaps revealing the top 30 bacterial families
 602 (%); (E-H) Bacterial genera with a mean relative abundance >1. Replicates are labelled with the numbers 1 to 15 for animals and 1 to 3 for seawater.

603



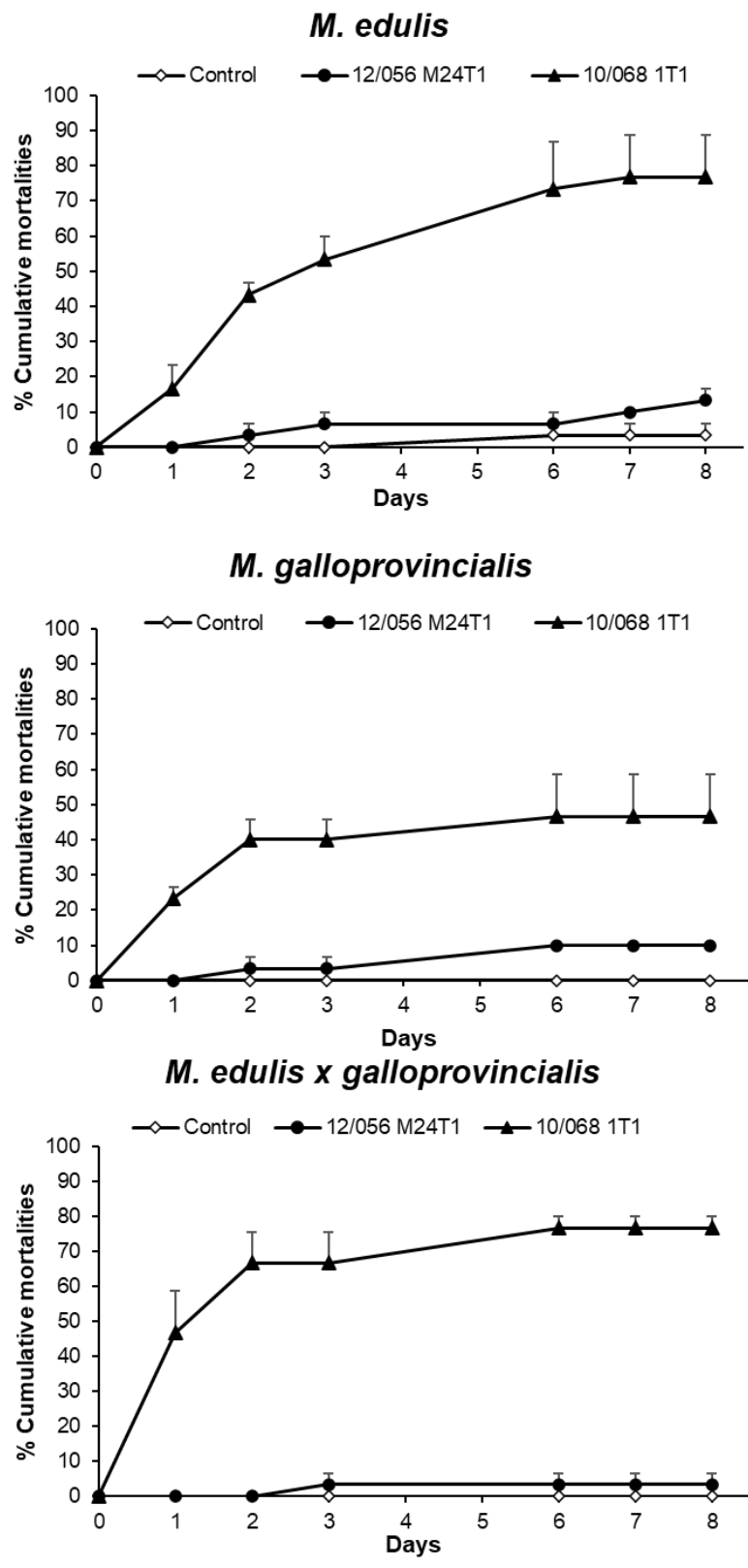
604

605 **Figure 3. Clustering of hosts and bacterial families.** (A) Representation of the association between hosts (rows) and bacterial families (columns)
606 as coloured vs. white rectangles. Colours are associated to the four different host types (purple: *M. edulis*, blue: *M. galloprovincialis*, green: hybrids,
607 yellow: seawater). The solid black lines (vertical and horizontal) delimit the clustering obtained using the latent block model, e.g. hosts (rows)
608 enclosed by the same horizontal lines belong to the same host cluster. (B) Alluvial plot linking the individual hosts within the five host clusters
609 (from cluster 1 at the bottom to cluster 5 at the top, on the left-hand side) with their host type (on the right-hand side).



610

611 **Figure 4. Resistance of *Mytilus* sp. to *V. splendidus* infection.** Mussels were intramuscularly injected with
 612 the pathogenic *V. splendidus* 10/068 1T1 or with the non-virulent *V. splendidus* 12/056 M24 T1 or with FSSW
 613 (control). Curves are mean of cumulative mortalities in triplicate tanks (10 mussels/tank) \pm SEM.



615 **Supplemental Figure S1. Relative abundance of ZymoBIOMICS microbial standards at the genus level.**

616 ZymoBIOMICS kit theoretically contains 8 bacterial strains: *Pseudomonas aeruginosa*, *Escherichia coli*,
617 *Salmonella enterica*, *Lactobacillus fermentum*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Listeria*
618 *monocytogenes*, *Bacillus subtilis*.

619 **Supplemental Figure S2. Alpha and beta diversity in mussels and seawater.** (A) alpha diversity expressed
620 as Chao1 and Shannon index. Data are the mean \pm SEM (n=15 for mussels, n=3 for seawater); (B) Beta
621 diversity calculated using Principal Component Analysis (PCoA) applied on Bray-Curtis distance matrix.

622 **Supplemental Figure S3. Uncertainty of cluster assignments.** Violin plots representing the distribution of
623 probabilities of assignment of each host (left panel) and bacterial family (right panel) to its LBM cluster (i.e.
624 the highest among all the probabilities of cluster memberships for each host and family). The white circle
625 indicates the value of the median of the distribution.

626 **Supplemental Table S1.** Number of pseudoreads in mussels and seawater before and after chimera filtering.

627 **Supplemental Table S2.** Mean relative abundance of the top 30 bacterial families identified in mussels and
628 seawater, n = 15 for mussels and n = 3 for seawater. Data are percentage \pm SEM.

629 **Supplemental Table S3.** Mean absolute abundance of *Vibrio*, *Cobetia* and *Arcobacter* species identified in
630 mussels, n = 15 for mussels. Data are mean \pm SEM. Only mean abundance > 100 OTUs in at least one mussel
631 species is considered.

632 **Supplemental Table S4.** Matrix summarizing the estimated probabilities of association between hosts and
633 bacterial families according to the LBM clusters they belong to. For instance, associations between a random
634 host from host cluster 3 and a random family from microbial cluster 4 occur with a probability of 7.88%.

635 **Supplemental Table S5.** Inventory of the bacterial families found in each of the four latent block model
636 clusters for bacteria.

637