



**HAL**  
open science

## Characterization of the marine aquaculture microbiome: A seasonal survey in a seabass farm

Roxane Roquigny, Julia Mougin, Cédric Le Bris, Maryse Bonnin-Jusserand,  
Périne Doyen, Thierry Grard

► **To cite this version:**

Roxane Roquigny, Julia Mougin, Cédric Le Bris, Maryse Bonnin-Jusserand, Périne Doyen, et al..  
Characterization of the marine aquaculture microbiome: A seasonal survey in a seabass farm. *Aqua-*  
*culture*, 2021, 531, pp.735987. 10.1016/j.aquaculture.2020.735987 . hal-03492676

**HAL Id: hal-03492676**

**<https://hal.science/hal-03492676v1>**

Submitted on 17 Oct 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License



26 that biofilms may be a potential reservoir in case of dysbiosis. This study helps better understand  
27 microbial interactions, especially with Vibrionaceae in aquaculture environments.

## 28 **1. Introduction**

29 Over the past decade, aquaculture mass production has increased worldwide (Ahmed et al., 2019).  
30 European seabass (*Dicentrarchus labrax*, L.) is one of the most farmed aquaculture species produced  
31 with a production about 191,000 t in 2016 (FAO, 2005-2020). Aquaculture is a fast-growing sector  
32 whose growth is limited by several challenges. There are geographic factors, such as the availability of  
33 water and land, as well as environmental factors such as the pollution of coastal zones (Ahmed et al.,  
34 2015). Disease outbreaks remain one of the main challenges, responsible for severe fish losses, leading  
35 to the loss of billions of dollars every year (Lafferty et al., 2015).

36 Belonging to Gammaproteobacteria, the Vibrionaceae family is composed of six well-identified  
37 genera and more than 140 species (Thompson et al., 2004). It is one of the most predominant bacterial  
38 groups in marine environments with a worldwide distribution (Ina-Salwany et al., 2019, Thompson et  
39 al., 2004). Several Vibrionaceae species have been identified as pathogens of farmed aquatic animals.  
40 Specifically, the *Vibrio*, *Photobacterium* and *Aliivibrio* genera are responsible for vibriosis (Ina-  
41 Salwany et al., 2019). Lethargy, skin lesions and hemorrhagic septicemia characterize affected fish  
42 (Ina-Salwany et al., 2019). On the one hand, global warming stimulates the occurrence of the repeated  
43 vibriosis outbreaks in farming systems (Travers et al., 2009, Vezzulli et al., 2015). On the other hand,  
44 biofilm formation enhances the virulence and the persistence of Vibrionaceae and some other species,  
45 partly due to horizontal gene transfer of potential virulence genes (Abe et al., 2020, Faruque et al.,  
46 2006, Karunasagar et al., 1996). Michaud et al. (2009) found that most of the detected potential  
47 pathogens (e.g. *Vibrio* spp., *Erwinia* spp. and *Coxiella* spp.) are associated with the biofilter biofilms  
48 in a recirculating aquaculture system (RAS) for rearing seabass. Multispecies biofilms are therefore  
49 considered as a reservoir for potential pathogenic and antibiotic-resistant bacteria (King et al., 2004).  
50 Moreover, Kotob et al. (2016) indicated that the relationships between microorganisms determine  
51 whether opportunistic pathogens proliferate and become virulent. Next-generation sequencing (NGS)

52 technologies are one way to consider the importance of all species for a better understanding of the  
53 development of disease outbreaks and the fragile equilibrium within microbiomes.

54 Despite the development of aquaculture, most studies still mainly focus on microbiota of the reared  
55 species' organs (Larsen et al., 2014). One study investigating the microbiome of the shrimp gut and  
56 tank water samples from two shrimp farms showed that microbial diversity was lower in the shrimp  
57 gut than in the tank water (Md Zoqratt et al., 2018). Another recent study investigated the skin and gill  
58 microbiomes of seabass and seabream from a fish farm (Rosado et al., 2019) and attested that the  
59 diversity of microbiomes was tissue-specific within and between species. Nevertheless, both studies  
60 detected potential pathogens such as *Vibrio* spp., which can lead to disease outbreaks in case of a shift  
61 in bacterial abundance (e.g. dysbiosis). Studies dealing with the microbial composition from biofilms  
62 and water in the tanks mainly involve RAS (Michaud et al., 2009, Wang et al., 2018, Wietz et al.,  
63 2009), but other studies have investigated temporal variations of bacterioplankton and the seasonal  
64 dynamics of bacterial and microeukaryotic plankton communities, e.g. in a semi-intensive European  
65 seabass aquaculture system (Duarte et al., 2019, Martins et al., 2018). The seasonal studies showed  
66 that changes in environmental variables influence the overall microbial communities.

67 In this context, we conducted a seasonal survey from April to October 2018 in a seabass farm in the  
68 North of France. Every year, this farm undergoes economic losses due to vibriosis outbreaks during  
69 summer. The aim of this study was to characterize the marine fish farm microbiomes and to determine  
70 the dynamics of bacterial populations in the different compartments of the aquaculture infrastructure.  
71 The objective was particularly to understand if *Vibrio* species get into the tanks during summer  
72 through the water inlets or if they persist throughout the year as biofilms. Studying the microbial  
73 composition can help understand the recurrence of vibriosis during summer. We therefore sampled  
74 biofilms and the water from the tanks and the inlets. The V4-V5 variable region of the 16S rRNA gene  
75 was amplified and then sequenced using an Illumina MiSeq platform. To our knowledge, this is the  
76 first study to carry out a weekly seasonal survey for 7 months on a fish farm with a comparison of  
77 microbial composition between water inlets, tank water and biofilms in the tanks.

## 78 **2. Materials and methods**

## 79 **2.1. Sampling campaign**

80 The experiments were conducted from April to October 2018 for 31 weeks, in Aquanord Ichthus sea  
81 farm (Gravelines, France). The fish farm installation and process have been described in the year 2018  
82 IFREMER annual report (Antajan et al., 2019). Briefly, the fish farm is a flow-through system where  
83 the tanks are supplied by two water inlets. The “cold-water” inlet supplies tanks with water from the  
84 surrounding local seawater. The “hot-water” inlet supplies tanks with seawater coming from the hot-  
85 water discharge canal of a nuclear power system. Several parameters, such as temperature, salinity,  
86 conductivity, pH and dissolved oxygen, were measured in the upper surface of the water of each  
87 compartment (Table S1). During the sampling campaign, no acute vibriosis outbreak occurred and fish  
88 losses remained low and stable. Fishes had been vaccinated with an autovaccine, as per annual routine,  
89 to fight against vibriosis. Fish density fluctuated from 20,000 to 30,000 individuals per breeding tank.  
90 The water capacity of each tank was 75 m<sup>3</sup>, with a water-column of 1.3 m. The tanks had a mean fish  
91 load of 48.13 kg of fish.m<sup>-3</sup>. Every week, 1 L of water was collected 20 cm below the water surface, in  
92 a sterilized bottle, in triplicate from the three tanks and the two water inlets. The 15 water bottles were  
93 kept on ice during the transport to the laboratory and filtered within 2 h. Nitrocellulose filters of 0.45  
94 µm pore size (Sartorius, Goettingen, Germany) were used and then frozen at -80°C in Luria-Bertoni  
95 (LB) broth medium supplemented with NaCl (LBS)/20% glycerol. The filtration stage has been  
96 optimized for maximum recovery of the bacterial community using a larger volume of water and  
97 larger pore size. The pore size was determined based on the high turbidity of the tank water due to  
98 suspended organic material. Moreover, to mimic the biofilm formation occurring on the walls of  
99 aquaculture tanks, we used the same concrete material to manufacture the pieces of concrete that were  
100 immersed (20 cm below the surface of the water), collected and brought back to the laboratory. Three  
101 pieces of concrete were not immersed and were considered as blanks (hereafter named B0), whereas  
102 samples immersed 2 h in the tanks were named 0B. Every two weeks, pieces of concrete were  
103 collected in triplicate from the three same tanks as the water samples. The nine pieces of concrete, still  
104 immersed in the water, were maintained on ice during the transport and until processing at the  
105 laboratory. Each piece of concrete was swabbed on a defined surface (of 2.34 cm<sup>2</sup>) with two cotton

106 swabs as previously done by Mougín et al. (2019) and swabs were stored at -80°C in an LBS/20%  
107 glycerol medium.

## 108 **2.2. DNA extraction**

109 Filter and swab samples were thawed and vortexed for 3 min to retrieve bacteria. Filters and swabs  
110 were discarded and the samples were centrifuged at 8000 × g for 10 min. Supernatants were removed  
111 and 25 mL or 3 mL of physiological water were added for samples from water or biofilm,  
112 respectively. After a brief vortex, the samples were centrifuged again (8000 × g for 10 min) and  
113 supernatants were removed. The pellets were resuspended in 2 mL of physiological water, and briefly  
114 vortexed. Samples were then centrifuged (8000 × g for 10 min) for the third time and the supernatants  
115 were discarded.

116 The DNA from each pellet was extracted using the DNeasy PowerBiofilm Kit (Qiagen, Hilden,  
117 Germany) following the manufacturer's protocol. The DNA purity was assessed on a Denovix DS-11  
118 spectrophotometer (Clinisciences, Nanterre, France) and DNA concentration was measured using the  
119 Qubit™ dsDNA HS Assay Kit on a Qubit 3.0 fluorometer (Invitrogen, Carlsbad, CA, USA). The  
120 quantity of extracted DNA was normalized to 5 ng·μL<sup>-1</sup> in Tris HCL (10 mM, pH 8.5) for the  
121 subsequent PCR assays and stored at -20°C. The 477 aliquots and the three negative control samples  
122 (composed of nuclease-free water) were used for metagenomic 16S rRNA gene sequencing. Hereafter  
123 samples were named tank water (W), cold-water inlet (CW), hot-water inlet (HW) and biofilm (B)  
124 samples depending on their compartment of origin. In the results, the sampling week is indicated  
125 before the compartment letter, e.g. sample named 8B came from swabbed biofilms at week 8.

## 126 **2.3. PCR amplification, library preparation, 16S rRNA gene amplicon sequencing**

127 A two-step PCR was performed according to 16S metagenomic sequencing library preparation  
128 protocol from Illumina. The first PCR step was carried out in duplicate. The amplicon primers 515F-  
129 Y/926R targeted the V4-V5 variable region of the 16S rRNA gene and were purchased from TIB  
130 MOLBIOL (Syntheselabor GmbH, Germany). The primers coupled with Illumina Reads adapters  
131 515F-Y 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA-  
132 3' and 926R 5'-

133 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCGYCAATTYMTTTRAGTTT-3'  
134 resulted in an amplicon of around 480 bp (Parada et al., 2016, Quince et al., 2011). PCR reactions  
135 were carried out in a final volume of 25  $\mu\text{L}$  using 0.5  $\mu\text{L}$  of each amplicon primer (10  $\mu\text{M}$ ), 2.5  $\mu\text{L}$  of  
136 DNA (5  $\text{ng}\cdot\mu\text{L}^{-1}$ ) and 12.5  $\mu\text{L}$  of 2X Platinum<sup>TM</sup> Green Hot Start Master Mix (Life Technologies SAS,  
137 Thermofisher Scientific, Villepinte, France). The PCR program was as follows: 94°C for 2 min  
138 followed by 30 cycles of a 94°C denaturation step for 45 s, a 50°C annealing step for 60 s and a 72°C  
139 elongation step for 90 s (Raut et al., 2018).

140 Dual indices were attached during the second step PCR, using the Illumina Nextera XT Index Kits v2  
141 (Illumina, Paris, France). PCR reaction was carried out in a final volume of 50  $\mu\text{L}$  using 5  $\mu\text{L}$  of  
142 previously purified amplicon, 5  $\mu\text{L}$  of each Nextera XT Index and 25  $\mu\text{L}$  of 2X Platinum<sup>TM</sup> Green Hot  
143 Start Master Mix. PCR program started with 94°C for 2 min followed by 8 cycles with a denaturation  
144 step of 94°C for 45 s, an annealing step of 59°C for 60 s and an elongation step of 72°C for 90 s. After  
145 each PCR step, PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter,  
146 Life Sciences, Villepinte, France) and were verified using electrophoresis on a 1% agarose gel. The  
147 expected amplicon size was around 500 bp long. Final product purity was assessed with a Denovix  
148 Spectrophotometer and final product concentration was measured using the Qubit<sup>TM</sup> dsDNA HS Assay  
149 Kit on a Qubit 3.0 fluorometer. The quantity of each sample was normalized to 4 nM in TrisHCL  
150 (10mM, pH 8.5) and then 5 $\mu\text{L}$  of diluted DNA from each library were mixed.

151 Following the Illumina protocol, pooled library was denatured, diluted to a final concentration of 4  
152 pM, combined with 10% PhiX (Illumina) and loaded using MiSeq reagent v3 kits (600 cycles, 2  $\times$  300  
153 bp) as recommended by the manufacturer. Finally, five sequencing runs of 96 samples each were  
154 sequenced as paired-end reads using an Illumina MiSeq platform (Illumina).

#### 155 **2.4. Sequence analyses**

156 Sequences were generated in demultiplexed fastq files and analyzed using the quantitative insights  
157 into microbial ecology 2 (QIIME2; release 2019.10) open-source platform (Bolyen et al., 2019,  
158 Caporaso et al., 2010). In each run, a total of 47,746,767 sequences were processed using DADA2  
159 pipeline (Callahan et al., 2016, Martin, 2011). Briefly, paired-end sequences were trimmed to 240 bp,

160 filtered, denoised, merged and chimeras were discarded, resulting in 20,134,001 dereplicated  
161 sequences (42.2%), (Table S2). The DADA2 pipeline gave five amplicon sequence variant (ASV)  
162 tables, which listed the number of times each exact ASV was observed in each sample (Callahan et al.,  
163 2017). The five tables were merged and technical triplicates grouped using the sum function. The  
164 combined table was filtered to exclude singletons and technical negative control samples. Taxonomy  
165 was assigned using a naive Bayes classifier trained on the 99\_otus SILVA 132-release database  
166 (Altschul et al., 1990, Bokulich et al., 2018, Edgar, 2010, Pedregosa et al., 2011, Quast et al., 2012,  
167 Wang et al., 2007). The table with taxonomic assignments was filtered a second time to exclude  
168 sequences assigned with mitochondria and chloroplast identification. Thus, analyses were  
169 subsequently performed on 159 samples, with 17,243,983 sequences clustered into 147,640 ASVs.  
170 Then, we chose a rarefaction depth of 29,725 sequences (27.41% of total retained sequences), which  
171 was the lowest read number. All samples were included in the beta-diversity analysis, without need to  
172 favor the rarest sequences. The values of the alpha-diversity indicators are detailed in Table S3. Chao1  
173 and Shannon indices were selected to evaluate richness and evenness across sampling weeks and  
174 compartments (Chao, 1984, Shannon, 1997). Two phylogenetic measures of beta-diversity were also  
175 selected and visualized in EMPEROR (Vázquez-Baeza et al., 2013). The unweighted UniFrac distance  
176 was used as a qualitative measure based on the presence/absence of different considered taxa. The  
177 weighted UniFrac distance was used as a quantitative measure based on the relative abundance of taxa  
178 (Lozupone and Knight, 2005, Lozupone et al., 2011). The phylogeny pipeline was previously applied  
179 to the data using MAFFT (Kato and Standley, 2013), MASK (Lane, 1991) and FastTree (Price et al.,  
180 2010) algorithms. Alpha-diversity statistical significances were tested using a Kruskal-Wallis test for  
181 pairwise comparisons (Kruskal and Wallis, 1952). Statistical significance of beta-diversity distances  
182 was analyzed using a PERMANOVA test (999 permutations).

### 183 **2.5. Data availability**

184 All sequences generated in this study are available in the NCBI sequence read archive under the  
185 Bioproject accession number PRJNA613386.

## 186 **3. Results**



### 187 **3.1. Sequencing and classification**

188 During the weekly sampling campaign from April to October 2018, total DNA from 477 samples from  
189 water or biofilm samples were extracted. Illumina MiSeq sequenced amplicons of the V4-V5 variable  
190 region of the 16S rRNA gene using the paired-end method and high quality sequences were obtained  
191 and processed. All technical triplicates were combined, leading to 159 samples subsequently analyzed  
192 and split into two datasets (Table S4). The water dataset was composed of 110 water samples from  
193 hot- and cold-water inlets and from the three breeding tanks for 22 weeks. The second dataset was  
194 composed of 48 biofilm samples from the three breeding tanks for 16 weeks and the non-immersed  
195 blank (B0). The mean number of sequences per sample obtained in biofilm samples was 140,286 and  
196 94,271 in water samples. The number of sequences ranged from 29,725 for the hot-water inlet sample  
197 at week 4 (4HW), to 315,298 for the biofilm sample from the second tank at week 24 (24B2). More  
198 than 17 million high quality sequences were thus processed with a mean of 108,453 sequences per  
199 sample. Each sequence was labeled, generating 147,640 ASVs. After a rarefaction process, taxonomy  
200 assignment was applied using the SILVA 132 database and the bacterial compositions were studied at  
201 different taxonomic levels.

### 202 **3.2. Bacterial composition**

#### 203 *3.2.1. Taxonomic identification*

204 At the phylum level (Fig. 1), results attributed 94.38% of microbial diversity, all sampling dates and  
205 compartments (biofilm or water samples) combined, to six major phyla: Proteobacteria, Bacteroidetes,  
206 Verrucomicrobia, Planctomycetes, Chloroflexi and Thaumarchaeota. Proteobacteria and Bacteroidetes  
207 represented more than 80% of the diversity with 54.52% and 26.85%, respectively. Moreover,  
208 Gamma-, Alpha- and Deltaproteobacteria were the three main classes composing the Proteobacteria  
209 phylum with 55.88%, 37.20% and 6.82%, respectively. Secondly, because the phylum taxonomic level  
210 did not distinguish among samples, we preferred the family taxonomic level. Thus, the taxonomy of  
211 water samples was assigned based on 1,291 ASVs at the family level, whereas biofilm samples were  
212 taxonomically identified from 1,009 ASVs (Table S4).

#### 213 *3.2.2. Composition across both datasets across time*

### 214 3.2.2.1. *Water dataset*

215 The samples from the water dataset were separated into three compartments: the cold-water inlet (Fig.  
216 2A), the hot-water inlet (Fig. 2B) and tank water samples (Fig. 3). The samples of the three tanks at  
217 the same sampling date for tank water were combined. No significant differences were observed  
218 between tanks at each week according to the pairwise comparisons of the diversity indices with the  
219 Kruskal-Wallis test. From April to October, bacterial compositions were obtained from 22 sampling  
220 weeks. The major phyla represented in water dataset were Proteobacteria, Bacteroidetes and  
221 Verrucomicrobia. Among them, Vibrionaceae, Rhodobacteraceae, Flavobacteriaceae and  
222 Rubritaleaceae were the most frequently observed families. The relative abundance of the bacterial  
223 families seemed more similar between cold- and hot- water inlets samples than with the tank water  
224 samples, particularly for Vibrionaceae. In fact, the relative abundance of Vibrionaceae barely reached  
225 5% in either water inlet sample (Fig. 2A, 2B), whereas it reached 50% in tank water samples, with the  
226 highest abundances for 18W, 24W and 28W (Fig. 3). Inversely, the relative abundance of  
227 Rhodobacteraceae and Flavobacteriaceae accounted for around 20% of the bacterial communities for  
228 each water inlet across time, and their abundance fluctuated more drastically in tank water samples  
229 from around 20% at week 0 to barely 10% at week 30. To a lesser extent, the SAR, Halieaceae,  
230 Desulfobulbaceae, Pirellulaceae and Cyanobiaceae families were specifically detected in samples from  
231 hot- and cold-water inlets and Colwelliaceae were mostly observed in tank water samples.

### 232 3.2.2.2. *Biofilm dataset*

233 As in the tank water samples, the biofilm samples from the three tanks at the same sampling date were  
234 combined. No significant differences were observed between tanks at each week according to the  
235 pairwise comparisons of the diversity indices using the Kruskal-Wallis test. The biofilm dataset was  
236 composed of the blank samples (three non-immersed pieces of concrete, named B0) and the samples  
237 from the tanks during 16 sampling weeks (Fig. 4). The blank B0 was mostly represented by specific  
238 phyla and families that were not identified in the other samples. The Actinobacteria, Firmicutes and  
239 Cyanobacteria phyla and the Burkholderiaceae family were only detected in the B0 samples, and no  
240 Planctomycetes were identified. At week 0, after 2 h immersion, the 0B biofilm sample already  
241 harbored more Flavobacteriaceae (20.38%), Vibrionaceae (14.84%), Rhodobacteraceae (7.02%),

242 Colwelliaceae (2.74%) and Saprospiraceae (4.30%) than the B0 sample. These relative abundances  
243 observed were quite similar to the proportions observed in 0W, i.e. in the tank water samples from the  
244 same sampling week. Proteobacteria, Bacteroidetes and Verrucomicrobia were represented in the  
245 biofilm dataset, as in the water dataset. Results also brought to light the presence of Planctomycetes,  
246 Chloroflexi and Thaumarchaeota. Among these phyla, Flavobacteriaceae, Rhodobacteraceae,  
247 Saprospiraceae, Rubritelaceae, Pirellulaceae, Vibrionaceae, Haliaceae and Rubinisphaeraceae were  
248 the most common families observed across time in biofilm samples. Three other families,  
249 Nitrosomonadaceae (from less than 0.01% in 2B to 1.71% in 30B), Nitrosopumilaceae (from less than  
250 0.01% in 2B to 8.14% in 30B) and Nitrospiraceae (from less than 0.01% in 2B to 1.20% in 30B) were  
251 increasingly present over time at the expense of the Flavobacteriaceae (from 37.89 % in 2B to 23.23 %  
252 in 30B). All together, the phyla, Planctomycetes, Chloroflexi and Thaumarchaeota appeared to be  
253 specific to biofilm samples and their relative abundance increased over time.

### 254 **3.3. Analyses of alpha-and beta-diversity**

#### 255 *3.3.1. Comparison of alpha-diversity indices*

256 Significant differences were always observed when comparing mean values of alpha-diversity indices  
257 between datasets. The biofilm dataset had significantly the greatest diversity than the water dataset  
258 both with the richness indicator Chao1 ( $H=13.87$ ,  $p\text{-value} < 0.001$ ) and the richness/evenness indicator  
259 Shannon ( $H=70.32$ ,  $p\text{-value} < 0.001$ ).

260 More specifically, there were no differences in richness between the bacterial communities of tank  
261 water and biofilm samples, whereas the latter were significantly richer than samples from either water  
262 inlet ( $p\text{-value} < 0.001$ , Fig. 5A). The Chao1 values revealed that tank water samples harbored as many  
263 bacterial communities as biofilm samples, but both compartments had more bacterial communities  
264 than either water inlet. Shannon's index values were significantly different between biofilm, water  
265 inlet and tank water samples ( $p\text{-value} < 0.001$ , Fig. 5B), this latter compartment being also  
266 significantly different from cold-water inlet samples ( $p\text{-value} < 0.05$ , Fig. 5B). Hot-water inlet samples  
267 did not show differences with tank water samples or cold-water inlet samples. Shannon's index, based  
268 on richness and evenness, estimated that the microbiome was richer in biofilm samples than in other

269 compartments, meaning that there were more bacterial communities and their presence was more  
270 evenly distributed. The bacterial communities were also richer and more evenly distributed in the tank  
271 water samples than in the cold-water inlet samples.

272 Some significant differences were observed when comparing the mean values of Shannon's index  
273 between sampling weeks within tank water samples or biofilm samples. Concerning tank water  
274 samples (Fig. 5C, p-value < 0.05), at weeks 16, 19 and 26 (16W, 19W, 26W Fig. 3), Shannon's index  
275 values showed that the bacterial communities in tank water samples were more diverse and evenly  
276 distributed than at weeks 2 and 28 (2W, 28W, Fig. 3). Regarding the biofilm samples (Fig. 5D, p-  
277 value < 0.05), except for week 2 (2B, Fig. 4), none of the bacterial communities were significantly  
278 different regarding their richness and evenness.

279 Alpha-diversity results indicated that the bacterial communities were more numerous and evenly  
280 distributed within the biofilm samples than in the tank water samples. Both communities from biofilm  
281 and tank water samples were more numerous and evenly distributed than communities from either  
282 water inlet.

### 283 3.3.2. Comparison of beta-diversity

284 We used a principal coordinate analysis (PCoA) to compare each microbiome sample with every other  
285 sample in each dataset (water and biofilm) and each compartment (hot- and cold-water inlets, tank  
286 water and biofilm). The qualitative comparison of microbiomes (Fig. 6A) based on presence or  
287 absence of considered taxa showed that two major axes explained almost 15% of the beta-diversity of  
288 all samples. Along these axes, samples from both water inlets could be grouped together, as well as  
289 biofilm samples on one end and tank water samples on the other end. The weighted UniFrac distance  
290 greatly increased the ability to discriminate samples by compartment (Fig. 6B). Moreover, the two  
291 main axes accounted for almost 50% of the variance. PERMANOVA analyses for both beta-diversity  
292 UniFrac distances tested if the hypothesis that one compartment was different from another was  
293 correct. Thus, compartments were statistically different between another by both the unweighted ( $R^2 =$   
294 0.14,  $p < 0.001$ ) and the weighted UniFrac ( $R^2=0.46$ ,  $p < 0.001$ ) distances.

295 The comparison between both UniFrac distances on the same data showed that the relative abundance  
296 of taxa in our samples was more compartment-discriminating than the presence/absence of different  
297 considered taxa, whereas presence/absence better dissociated the samples from each other.

### 298 **3.4. Detection and identification of the Vibrionaceae family**

299 The study of the distribution of the Vibrionaceae family was performed on sequences identified by  
300 genus within this taxonomic level. Almost 15% of all sequences were identified as Vibrionaceae,  
301 namely 2,575,816 sequences (Table S4), 90% of which (2,343,502 sequences) came from the water  
302 dataset. Six different already identified genera and two other groups defined as uncultured and  
303 unassigned were determined within the Vibrionaceae family (Table 1). *Vibrio* spp. (1,796,843  
304 sequences), *Photobacterium* spp. (404,774 sequences), *Aliivibrio* spp. (328,413 sequences) and  
305 *Enterovibrio* spp. (29,895 sequences) were the most identified ASVs. Moreover, Shannon's index was  
306 applied to Vibrionaceae within the tank water samples (Fig. 7). Significant differences were observed  
307 for 19W and 20W samples (both in August) compared with samples from 0W to 10W (except 6W)  
308 and from 26W to 30W. During these two weeks in August, the mean temperature was 20.5°C (+/-  
309 0.19°C) and pH ranged from 7.24 to 7.40 (Table S1).

## 310 **4. Discussion**

311 Here, we conducted a seasonal survey in the North of France, from April to October 2018 in a seabass  
312 farm that undergoes repeated summer outbreaks of vibriosis. Although no acute vibriosis outbreak  
313 occurred during this survey, the characterization of the microbiomes from the marine aquaculture  
314 environment was of paramount importance to evaluate and monitor the bacterial communities and  
315 their potential interactions over time. Specifically, the characterization of the Vibrionaceae population  
316 dynamics in the various aquaculture compartments shed light on which compartments (water inlets,  
317 water tank and biofilm) may be a source or a reservoir of Vibrionaceae. Using Illumina MiSeq  
318 technology targeting the V4-V5 variable region of the 16S rRNA gene, we detected and identified the  
319 bacterial communities through a weekly sampling campaign. The presence of Vibrionaceae was  
320 ubiquitous and detected in all samples (Table 1). Despite a common microbiome shared between all

321 samples, alpha-diversity indices were significantly different between sample compartments (Fig. 5A,  
322 5B).

#### 323 **4.1. Common microbiome composition**

324 A common microbiome was detected in samples from all compartments. This commonality is likely  
325 due to the source of the water used to supply the tanks in water and in which the pieces of concrete  
326 were immersed. Taking all the results together, the Proteobacteria (54.52 %) and Bacteroidetes (26.85  
327 %) phyla formed the main components of the microbiomes from the water and biofilm compartments  
328 (Fig. 1). These phyla have already been detected in a seabass farm (Duarte et al., 2019) and were also  
329 predominant in the seabass skin microbiome (Chiarello et al., 2015, Pimentel et al., 2017, Rosado et  
330 al., 2019). Moreover, Gamma-, Alphaproteobacteria and Bacteroides appear to be the first classes to  
331 form a biofilm in a RAS (Wietz et al., 2009).

332 A core microbiome analysis (data not shown) revealed that Vibrionaceae (*Vibrio* genus, Table 1),  
333 Rhodobacteraceae (with the *Loklanella*, *Sulfitobacter*, *Planktotalea* genera), Rhizobiaceae,  
334 Flavobacteriaceae and Saprospiraceae were the families (belonging to the Proteobacteria and  
335 Bacteroidetes phyla) shared among all samples. These results were also consistent with those of Wang  
336 et al. (2018), who found that genera from the Saprospiraceae, Flavobacteriaceae and  
337 Rhodobacteraceae families were shared between RAS tank water and the RAS biofilter. Moreover,  
338 Flavobacteriaceae and Rhodobacteraceae have also been reported in association with zooplankton,  
339 notably because of their respective capacities to participate in the carbon cycle by degrading high-  
340 molecular-weight organic matter, and to contribute to biofilm formation (De Corte et al., 2018).

#### 341 **4.2. Overall microbiota differences between sampling compartments**

342 The alpha-and beta-diversity indices used in this study (Chao1, Shannon, unweighted and weighted  
343 UniFrac distances) highlighted differences in the bacterial composition between the compartments and  
344 between samples within each compartment (Fig. 5, Fig. 6). The biofilm samples and tank water  
345 samples harbored the most diverse bacterial populations compared with the water inlet samples (Fig.  
346 5A, 5B). These observations could be partly explained by the presence of seabass in the tanks. The  
347 Colwelliaceae family was more abundant in the tank water samples than in the water inlet samples

348 (Fig. 2, Fig. 3). Pimentel et al. (2017) highlighted that the accumulation of uneaten feed in the tank  
349 generate anoxic areas, where the genus *Colwellia* could be found and contribute to anaerobic  
350 processes. Otherwise, previous studies have shown that biofilm communities are distinct from other  
351 microbial communities, for example in raw water (Luo et al., 2013). The increasing presence of the  
352 Nitrosomonadaceae, Nitrospiraceae and Nitrosopumilaceae families appeared to be specific to the  
353 biofilm samples (Fig. 4). In fact, these three families are involved in nitrification processes and are  
354 known as ammonium-oxidizing bacteria and ammonium-oxidizing archaea (Rurangwa and Verdegem,  
355 2015). Luo et al. (2013) revealed that the presence of Firmicutes and *Nitrospiraes* spp. in microbial  
356 biofilms improves the quality of the raw water by dissolving oxygen and modifying nitrogen levels.  
357 Planctomycetes, such as Pirellulaceae (Fig. 4), also participate in ammonium oxidation in anaerobic  
358 conditions (Schreier et al., 2010). The presence of these bacterial populations in a biofilm is crucial  
359 because they oxidize ammonia and nitrite into less toxic compounds, such as nitrates (Rurangwa and  
360 Verdegem, 2015).

361 Moreover, our results showed that bacterial populations composing biofilm established between the 2  
362 hours immersion and the second sampling week. Bacterial communities on the non-immersed blank  
363 disappeared after just 2 h of immersion, as manifested by the Actinobacteria, Cyanobacteria and  
364 Firmicutes families. Firmicutes have been found in bacterial communities from RAS biofilters or in  
365 biofilms in raw water distribution systems (Luo et al., 2013, Schreier et al., 2010). These families have  
366 often been observed in various inhabited areas, such as utensils or on stones (Ng et al., 2016). The  
367 populations unique to biofilms changed rapidly, despite the presence and constancy of families  
368 common to all compartments. Our results showed that Planctomycetes, Chloroflexi and  
369 Thaumarchaeota phyla as being specific to biofilm samples.

370 During this seasonal campaign, the cold-water inlet underwent the most temperature changes (from  
371 9.08°C to 21.1°C), due to warmer waters in the summer, but no major differences in bacterial  
372 communities were observed in this compartment. The water temperature in the tanks was maintained  
373 approximately constant by mixing in variable proportions the hot- and cold-water inlets (from 17.97°C  
374 to 21.17°C). Nevertheless, the bacterial communities did not change with the slightly higher  
375 temperatures during summer (Fig. 5C, 5D). Moreover, a Spearman correlation test was performed

376 between all physical parameters (temperature, pH, oxygen, salinity and conductivity) measured in the  
377 tanks, the diversity indicators (Chao1 and Shannon from tank water and biofilm samples) and the  
378 relative abundance of Vibrionaceae (from the tank water and biofilm samples). No significant  
379 association was revealed between evenness and richness variations from the tank water and biofilm  
380 samples and temperature, oxygen, salinity or conductivity considered independently. A negative  
381 correlation was revealed only between pH and both diversity indicators in biofilm samples. Bacterial  
382 communities of biofilm samples appeared more diverse when the pH was lower. The pH variation  
383 ranged from 6.35 to 7.97 during the sampling campaign (Table S1). The pH decrease may be linked to  
384 the nitrification process occurring in biofilm samples. Finally, the relative abundance of Vibrionaceae  
385 from water tank samples correlated negatively with pH and oxygen levels in the tanks, whereas the  
386 relative abundance of Vibrionaceae from biofilm samples correlated positively with the temperature in  
387 the tanks. Then the temperature rising seemed promote Vibrionaceae proliferation in biofilm samples  
388 and their role as reservoir in case of dysbiosis.

#### 389 **4.3. Potential pathogens detected**

390 We then focused on the Vibrionaceae family in tank water samples, because this compartment  
391 contained more Vibrionaceae than the other compartments. Our results suggested that biofilms may be  
392 a reservoir of Vibrionaceae, and tank water seemed amenable to its proliferation. A lower Shannon  
393 index was indeed obtained (18W, 24W and 28W, Fig. 5C) when the Vibrionaceae were most abundant  
394 (18W, 24W and 28W, Fig. 3). The balance between bacterial communities was thus disturbed, because  
395 one bacterial population had proliferated in particular. Among the Vibrionaceae family, four genera  
396 were frequent, in order of importance: *Vibrio*, *Photobacterium*, *Aliivibrio* and *Enterovibrio*. More  
397 precisely, the species level determination identified *Vibrio ponticus*, *Vibrio aestivus*, *Vibrio*  
398 *sinaloensis* and *Vibrio anguillarum*, as well as *Photobacterium damsela* subsp. *damsela*,  
399 *Photobacterium swingsii*, *Photobacterium gaetbulicola* Gung47, *Photobacterium rosenbergii*, and  
400 *Photobacterium damsela* subsp. *piscicida*, and *Aliivibrio fischeri* and *Enterovibrio nigricans* (Table  
401 1). Most pathogenic *Vibrio* species, such as *Vibrio harveyi* or *Vibrio vulnificus*, were not identified,  
402 although we expected to find *V. harveyi* since it has already been identified as major pathogens in this



403 aquaculture infrastructure (Mougin et al., 2020). The Vibrionaceae population more than doubled  
404 during the sampling campaign (20% at 0W and 50% at 28W, Fig. 3). The peaks of diversity were  
405 reached at weeks 19 and 20 (during August), suggesting that all genera were present and in stable  
406 proportions (Fig. 7). Despite this variation, the equilibrium between genera remained relatively stable  
407 throughout the sampling weeks.

408 Our results revealed that the water from the tanks contained Vibrionaceae and may be sufficiently rich  
409 in bacteria to harbor pathogens and their antagonistic members. Some studies have indeed reported  
410 that *Roseobacter* spp. (family Rhodobacteraceae) may establish antagonistic activity against  
411 Vibrionaceae and particularly against *V. anguillarum* (Balcázar et al., 2010, Hjelm et al., 2004).  
412 Among the Flavobacteriaceae, *Polaribacter* is an opportunist common fish farm bacteria colonizing an  
413 already weakened fish and has already been detected in the core microbiome of seabass and seabream  
414 (Rosado et al., 2019). Our results showed that increases in Rhodobacteraceae and Flavobacteriaceae  
415 were associated with decreases in Vibrionaceae. The absence of acute vibriosis outbreaks during the  
416 campaign suggests that there is an equilibrium between pathogenic Vibrionaceae and the other  
417 antagonistic bacterial communities. It would be relevant to study the putative antagonistic activity  
418 between sampled Vibrionaceae, Rhodobacteraceae and Flavobacteriaceae. Simultaneously,  
419 identification and detection of biofilm microbiomes now makes it possible to investigate the capacity  
420 of horizontal gene transfer of the different species and to investigate antibiotic resistance. Despite the  
421 presence of potential pathogens, there were no disease outbreaks; therefore, the vaccination campaign  
422 appears to have worked. In the present study, no sampling was carried out directly on fish firstly for  
423 ethical reasons and secondly because the main objective was to consider the aquaculture  
424 compartments. Nevertheless, future studies should compare our results with fish microbiomes to  
425 confirm the efficiency of vaccination. Finally, our results demonstrated the importance of  
426 simultaneously studying all bacterial communities.

## 427 **5. Conclusion**

428 In conclusion, this is the first study to compare bacterial communities from several water  
429 compartments and from biofilms in an aquaculture farm over time with a seven-month weekly

430 sampling campaign. Proteobacteria and Bacteroidetes, which are common phyla in water ecosystems,  
431 were abundant. The diversity of bacterial communities differed within compartments, although there  
432 were no significant correlations with water quality parameters. Biofilm samples contained higher  
433 bacterial diversity than water samples and tank water samples were richer than samples from water  
434 inlets. The proportion of Vibrionaceae was high in tank water samples. All six genera composing the  
435 Vibrionaceae family were identified. The natural environment seemed to be a source of Vibrionaceae,  
436 which found a favorable environment to develop and then persist in biofilms in the aquaculture  
437 system. In the future, we plan to investigate the antagonistic effects between Vibrionaceae and other  
438 already present bacteria to design solutions such as probiotics use to monitor bacterial populations and  
439 promote sustainable aquaculture. Moreover, the aquaculture environment can be monitored using a  
440 metatranscriptomic analysis. The bacterial populations detected can be linked to the primary and  
441 secondary metabolic functions observed, such as virulence.

## 442 **Funding**

443 This work was supported by the French government, IFREMER and the Hauts-de-France Regional  
444 Council as part of the CPER 2018-2019 MARCO project.

445 This work was also supported by the Hauts-de-France Regional Council and ULCO, which provided  
446 funding for Julia Mougin's PhD studies.

## 447 **Declaration of competing interest**

448 The authors declare that they have no known competing financial interests or personal relationships  
449 that could have appeared to influence the work reported in this paper.

## 450 **Acknowledgements**

451 Authors wish to thank Aquanord-Ictus as a contributor to this project, especially Guillaume Tielie,  
452 Anabelle Duhamel and Céline Doyen. Authors want to thank Dominique Hervio-Heath for her advice  
453 on sequencing targets. We would also like to thank the Hauts-de-France Regional Council and ULCO  
454 (PhD funding for Julia Mougin); and the CPER 2014-2020 MARCO project (post-doctoral funding for  
455 Roxane Roquigny).

## 456 **References**

- 457 Abe K, Nomura N, Suzuki S. 2020. Biofilms: hot spots of horizontal gene transfer (HGT) in aquatic  
458 environments, with a focus on a new HGT mechanism. *FEMS Microbiol Ecol* 96:fiiaa31.
- 459 Ahmed N, Thompson S, Glaser M. 2019. Global aquaculture productivity, environmental  
460 sustainability, and climate change adaptability. *Environ Manage* 63:159-172.
- 461 Ahmed W, Staley C, Sadowsky MJ, Gyawali P, Sidhu JPS, Palmer A, Beale DJ, Toze S. 2015.  
462 Toolbox Approaches Using Molecular Markers and 16S rRNA Gene Amplicon Data Sets for  
463 Identification of Fecal Pollution in Surface Water. *Appl Environ Microb* 81:7067-7077.
- 464 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J*  
465 *Mol Biol* 215:403-410.
- 466 Antajan E, Devreker D, Loots C, Rocroy M, Alizier S. 2019. Surveillance écologique du site de  
467 production électronucléaire de Gravelines, novembre 2017 – octobre 2018. Ifremer  
468 RODE/LITTORAL/LER/ BL19.02:208 p.
- 469 Balcázar JL, Lee NM, Pintado J, Planas M. 2010. Phylogenetic characterization and in situ detection  
470 of bacterial communities associated with seahorses (*Hippocampus guttulatus*) in captivity. *Syst Appl*  
471 *Microbiol* 33:71-77.
- 472 Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory  
473 Caporaso J. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with  
474 QIIME 2's q2-feature-classifier plugin. *Microbiome* 6:90.
- 475 Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ,  
476 Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT,  
477 Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC,  
478 Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM,  
479 Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H,  
480 Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR,  
481 Keim P, Kelley ST, Knights D, et al. 2019. Reproducible, interactive, scalable and extensible  
482 microbiome data science using QIIME 2. *Nat Biotechnol* 37:852-857.

483 Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational  
484 taxonomic units in marker-gene data analysis. *ISME J* 11:2639-2643.

485 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-  
486 resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581-583.

487 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG,  
488 Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA,  
489 McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann  
490 J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community  
491 sequencing data. *Nat Methods* 7:335-336.

492 Chao A. 1984. Nonparametric estimation of the number of classes in a population. *Scand J Stat*  
493 11:265-270.

494 Chiarello M, Villéger S, Bouvier C, Bettarel Y, Bouvier T. 2015. High diversity of skin-associated  
495 bacterial communities of marine fishes is promoted by their high variability among body parts,  
496 individuals and species. *FEMS Microbiol Ecol* 91:fiv061.

497 De Corte D, Srivastava A, Koski M, Garcia JAL, Takaki Y, Yokokawa T, Nunoura T, Elisabeth NH,  
498 Sintes E, Herndl GJ. 2018. Metagenomic insights into zooplankton-associated bacterial communities.  
499 *Environ Microbiol* 20:492-505.

500 Duarte LN, Coelho FJRC, Cleary DFR, Bonifácio D, Martins P, Gomes NCM. 2019. Bacterial and  
501 microeukaryotic plankton communities in a semi-intensive aquaculture system of sea bass  
502 (*Dicentrarchus labrax*): A seasonal survey. *Aquaculture* 503:59-69.

503 Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*  
504 26:2460-2461.

505 FAO. 2005-2020. Cultured Aquatic Species Information Programme *Dicentrarchus labrax*. Cultured  
506 Aquatic Species Fact Sheets. [http://www.fao.org/fishery/culturedspecies/Dicentrarchus\\_labrax/en](http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en).  
507 Accessed Updated 18 February 2005.

508 Faruque SM, Biswas K, Udden SMN, Ahmad QS, Sack DA, Nair GB, Mekalanos JJ. 2006.  
509 Transmissibility of cholera: *In vivo*-formed biofilms and their relationship to infectivity and  
510 persistence in the environment. *P Natl Acad Sci USA* 103:6350-6355.

511 Hjelm M, Riaza A, Formoso F, Melchiorson J, Gram L. 2004. Seasonal Incidence of Autochthonous  
512 Antagonistic *Roseobacter* spp. and *Vibrionaceae* Strains in a Turbot Larva (*Scophthalmus maximus*)  
513 Rearing System. *Appl Environ Microb* 70:7288-7294.

514 Ina-Salwany MY, Al-saari N, Mohamad A, Mursidi F-A, Mohd-Aris A, Amal MNA, Kasai H, Mino  
515 S, Sawabe T, Zamri-Saad M. 2019. Vibriosis in Fish: A Review on Disease Development and  
516 Prevention. *J Aquat Anim Health* 31:3-22.

517 Karunasagar I, Otta SK, Karunasagar I. 1996. Biofilm formation by *Vibrio harveyi* on surfaces.  
518 *Aquaculture* 140:241-245.

519 Katoh K, Standley DM. 2013. MAFFT Multiple Sequence Alignment Software Version 7:  
520 Improvements in Performance and Usability. *Mol Biol Evol* 30:772-780.

521 King RK, Flick GJ, Pierson D, Smith SA, Boardman GD, Coale CW. 2004. Identification of Bacterial  
522 Pathogens in Biofilms of Recirculating Aquaculture Systems. *J Aquat Food Prod T* 13:125-133.

523 Kotob MH, Menanteau-Ledouble S, Kumar G, Abdelzaher M, El-Matbouli M. 2016. The impact of  
524 co-infections on fish: a review. *Vet Res* 47:98.

525 Kruskal WH, Wallis WA. 1952. Use of Ranks in One-Criterion Variance Analysis. *J Am Stat Assoc*  
526 47:583-621.

527 Lafferty KD, Harvell CD, Conrad JM, Friedman CS, Kent ML, Kuris AM, Powell EN, Rondeau D,  
528 Saksida SM. 2015. Infectious Diseases Affect Marine Fisheries and Aquaculture Economics. *Annu*  
529 *Rev Mar Sci* 7:471-496.

530 Lane DJ. 1991. 16S/23S rRNA sequencing, p 115-175. *In* E S, M G (ed), *Nucleic acid techniques in*  
531 *bacterial systematics*. John Wiley and Sons, Chicester, NewYork.

532 Larsen AM, Mohammed HH, Arias CR. 2014. Characterization of the gut microbiota of three  
533 commercially valuable warmwater fish species. *J Appl Microbiol* 116:1396-1404.

534 Lozupone C, Knight R. 2005. UniFrac: a New Phylogenetic Method for Comparing Microbial  
535 Communities. *Appl Environ Microb* 71:8228-8235.

536 Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. 2011. UniFrac: an effective distance  
537 metric for microbial community comparison. *ISME J* 5:169-172.

538 Luo J, Liang H, Yan L, Ma J, Yang Y, Li G. 2013. Microbial community structures in a closed raw  
539 water distribution system biofilm as revealed by 454-pyrosequencing analysis and the effect of  
540 microbial biofilm communities on raw water quality. *Bioresource Technol* 148:189-195.

541 Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads.  
542 *EMBnet Journal* 17:3.

543 Martins P, Coelho FJRC, Cleary DFR, Pires ACC, Marques B, Rodrigues AM, Quintino V, Gomes  
544 NCM. 2018. Seasonal patterns of bacterioplankton composition in a semi-intensive European seabass  
545 (*Dicentrarchus labrax*) aquaculture system. *Aquaculture* 490:240-250.

546 Md Zoqratt MZH, Eng WWH, Thai BT, Austin CM, Gan HM. 2018. Microbiome analysis of Pacific  
547 white shrimp gut and rearing water from Malaysia and Vietnam: implications for aquaculture research  
548 and management. *PeerJ* 6:e5826.

549 Michaud L, Lo Giudice A, Troussellier M, Smedile F, Bruni V, Blancheton JP. 2009. Phylogenetic  
550 characterization of the heterotrophic bacterial communities inhabiting a marine recirculating  
551 aquaculture system. *J Appl Microbiol* 107:1935-1946.

552 Mougín J, Copin S, Bojolly D, Raguénat V, Robert-Pillot A, Quilici M-L, Midelet-Bourdin G, Grard  
553 T, Bonnin-Jusserand M. 2019. Adhesion to stainless steel surfaces and detection of viable but non  
554 cultivable cells of *Vibrio parahaemolyticus* and *Vibrio cholerae* isolated from shrimps in seafood  
555 processing environments: Stayin' alive? *Food Control* 102:122-130.

556 Mougín J, Roquigny R, Travers M-A, Grard T, Bonnin-Jusserand M, Le Bris C. 2020. Development  
557 of a mreB-targeted real-time PCR method for the quantitative detection of *Vibrio harveyi* in seawater  
558 and biofilm from aquaculture systems. *Aquaculture*:735337.

559 Ng DHP, Kumar A, Cao B. 2016. Microorganisms meet solid minerals: interactions and  
560 biotechnological applications. *Appl Microbiol Biot* 100:6935-6946.

561 Parada AE, Needham DM, Fuhrman JA. 2016. Every base matters: assessing small subunit rRNA  
562 primers for marine microbiomes with mock communities, time series and global field samples.  
563 *Environ Microbiol* 18:1403-1414.

564 Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P,  
565 Weiss R, Dubourg V. 2011. Scikit-learn: Machine learning in Python. J Mach Learn Res 12:2825-  
566 2830.

567 Pimentel T, Marcelino J, Ricardo F, Soares AMVM, Calado R. 2017. Bacterial communities 16S  
568 rDNA fingerprinting as a potential tracing tool for cultured seabass *Dicentrarchus labrax*. Sci Rep UK  
569 7:11862.

570 Price MN, Dehal PS, Arkin AP. 2010. FastTree 2 – Approximately Maximum-Likelihood Trees for  
571 Large Alignments. PLOS One 5:e9490.

572 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012. The  
573 SILVA ribosomal RNA gene database project: improved data processing and web-based tools.  
574 Nucleic Acids Res 41:D590-D596.

575 Quince C, Lanzen A, Davenport RJ, Turnbaugh PJ. 2011. Removing Noise From Pyrosequenced  
576 Amplicons. BMC Bioinformatics 12:38.

577 Raut S, Polley HW, Fay PA, Kang S. 2018. Bacterial community response to a preindustrial-to-future  
578 CO<sub>2</sub> gradient is limited and soil specific in Texas Prairie grassland. Global Change Biol 24:5815-5827.

579 Rosado D, Pérez-Losada M, Severino R, Cable J, Xavier R. 2019. Characterization of the skin and gill  
580 microbiomes of the farmed seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*).  
581 Aquaculture 500:57-64.

582 Rurangwa E, Verdegem MCJ. 2015. Microorganisms in recirculating aquaculture systems and their  
583 management. Rev Aquacult 7:117-130.

584 Schreier HJ, Mirzoyan N, Saito K. 2010. Microbial diversity of biological filters in recirculating  
585 aquaculture systems. Curr Opin Biotech 21:318-325.

586 Shannon C. 1997. The mathematical theory of communication. 1963, p 306-317, MD Comput Comput  
587 Med Pract, vol 14.

588 Thompson FL, Iida T, Swings J. 2004. Biodiversity of Vibrios. Microbiol Mol Biol R 68:403-431.

589 Travers M-A, Basuyaux O, Le Goïc N, Huchette S, Nicolas J-L, Koken M, Paillard C. 2009. Influence  
590 of temperature and spawning effort on *Haliotis tuberculata* mortalities caused by *Vibrio harveyi*: an  
591 example of emerging vibriosis linked to global warming. Global Change Biol 15:1365-1376.

592 Vázquez-Baeza Y, Pirrung M, Gonzalez A, Knight R. 2013. EMPeror: a tool for visualizing high-  
593 throughput microbial community data. *Gigascience* 2:2047-217X-2-16.

594 Vezzulli L, Pezzati E, Brettar I, Höfle M, Pruzzo C. 2015. Effects of Global Warming on *Vibrio*  
595 Ecology. *Microbiol Spectrum* 3:VE-0004-2014.

596 Wang C, Sun G, Li S, Li X, Liu Y. 2018. Intestinal microbiota of healthy and unhealthy Atlantic  
597 salmon *Salmo salar* L. in a recirculating aquaculture system. *Chin J Oceanol Limn* 36:414-426.

598 Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian Classifier for Rapid Assignment of  
599 rRNA Sequences into the New Bacterial Taxonomy. *Appl Environ Microb* 73:5261-5267.

600 Wietz M, Hall MR, Høj L. 2009. Effects of seawater ozonation on biofilm development in aquaculture  
601 tanks. *Syst Appl Microbiol* 32:266-277.

602

603



## 604 **Figure Legends**

605 Figure 1: The six most abundant phyla (from all samples, over all sampling times), representing  
606 94.38% of all sequences. The number above each bar indicates the percentage of each phylum. The  
607 percentage of each class of Proteobacteria is shown in the blue donut chart.

608 Figure 2: Bar plots of relative abundance (%) of bacterial families throughout the 30 sampling weeks  
609 composing A) the cold-water inlet samples, B) the hot-water inlet samples. Bacterial families are  
610 grouped by phylum with the second color scale on the top. Families unassigned or that did not reach a  
611 minimum of 3% in at least one sample were grouped in the “Others” category.

612 Figure 3: Bar plots of relative abundance (%) of bacterial families composing the tank water samples  
613 throughout the 30 sampling weeks. Bacterial families are grouped by phylum with the second color  
614 scale on the top. Families unassigned or that did not reach a minimum of 3% in at least one sample  
615 were grouped in the “Others” category.

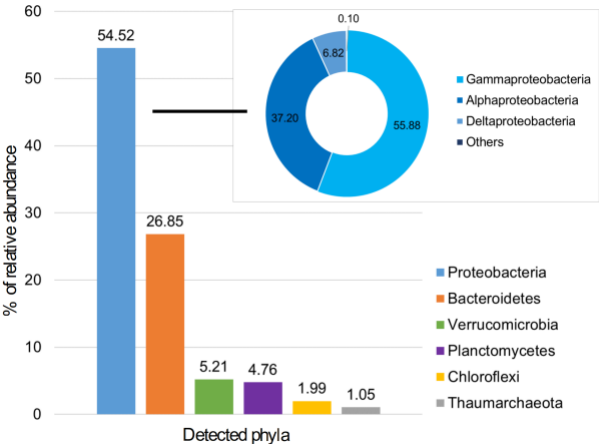
616 Figure 4: Bar plots of relative abundance (%) of bacterial families across biofilm samples across  
617 sampling weeks. Bacterial families are grouped by phylum and by family (color scale on the right).  
618 Unassigned families or those that did not reach a minimum of 1% in at least one sample were grouped  
619 in the “Others” category.

620 Figure 5: Mean values and standard deviations of Chao1 (A) and Shannon (B, C, D) alpha-diversity  
621 indices plotted by compartment (A, B) or by sampling week for tank water samples (C) and for  
622 biofilm samples (D). All purple and gray box plots respectively represent tank water samples and  
623 biofilm samples from the three breeding tanks. Blue and red box plots correspond to cold- and hot-  
624 water inlet samples, respectively. Kruskal-Wallis tests for pairwise comparisons were carried out and  
625 different letters indicate significant differences.

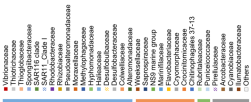
626 Figure 6: Beta-diversity of the 159 samples. (A) Unweighted UniFrac and (B) weighted UniFrac  
627 distances were analyzed and plotted using principal coordinates analysis (PCoA). Each point  
628 represents a microbiome sample (composed of 29,725 sequences corresponding to the rarefaction  
629 depth). Rings and stars distinguish the water dataset from the biofilm dataset, respectively. Blue and  
630 red colors correspond to cold- and hot-water inlet samples, respectively, whereas the three shades of  
631 purple indicate the water or biofilm samples from the three breeding tanks. The brown star indicates  
632 the B0 sample.

633 Figure 7: Mean values and standard deviations of Shannon alpha-diversity index of the Vibrionaceae  
634 family, plotted by sampling week for tank water samples. Kruskal-Wallis tests for pairwise  
635 comparisons were carried out and different letters indicate significant differences.

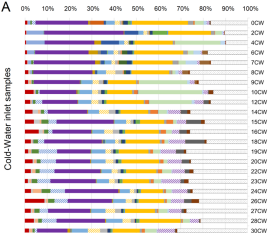
636



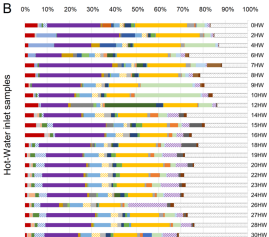
Proteobacteria Bacteroidetes Verrucomicrobia Other Phyla



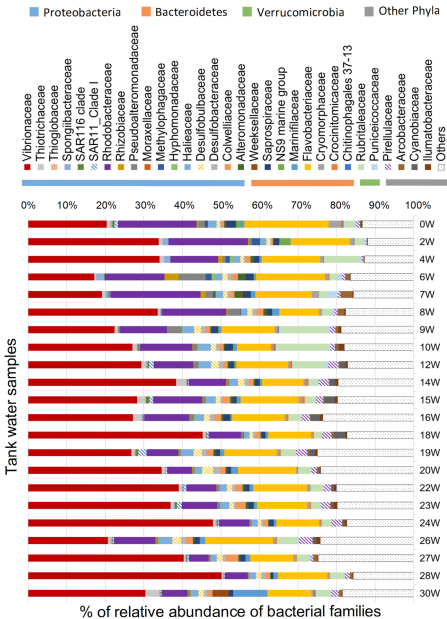
A



B

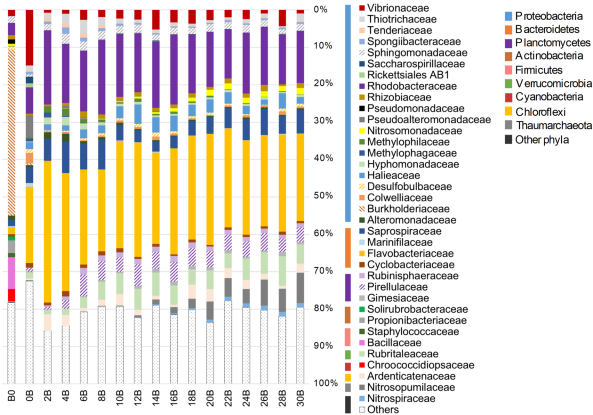


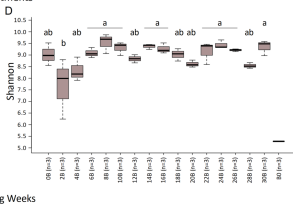
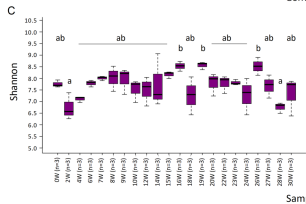
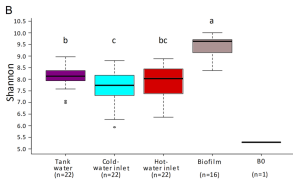
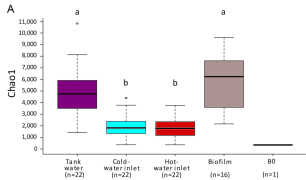
% of relative abundance of bacterial families

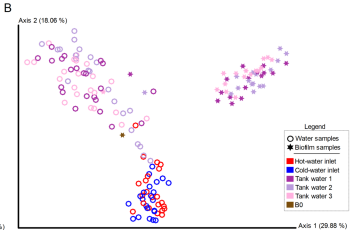
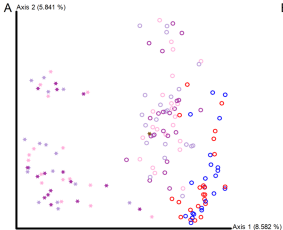


# Biofilm samples

% of relative abundance of bacterial families







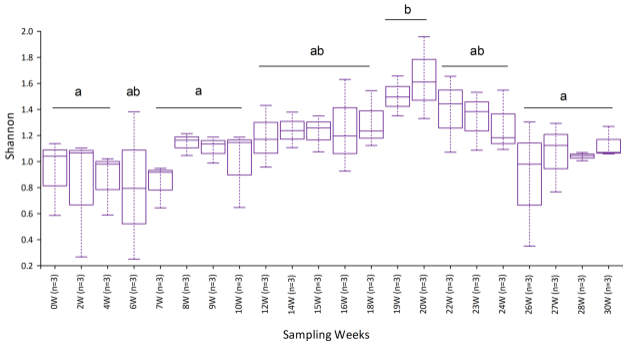




Table 1: Number of sequences identified, using the SILVA 132 database, in the Vibrionaceae family and number of samples

Taxonomy of Vibrionaceae detected						All samples		
phylum	class	order	family	genus	species	Number of sequences	Number of samples observed in	Number of total samples
Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	<b>Vibrio</b>		<b>1,796,843</b>	<b>159</b>	159
				—		1,750,394	159	-
				<i>Vibrio ponticus</i>		24,369	68	-
				<i>Vibrio aestivus</i>		16,467	20	-
				<i>uncultured bacterium</i>		2,803	67	-
				<i>Vibrio sinaloensis</i>		2,436	6	-
				<i>Vibrio anguillarum</i>		336	28	-
				<i>uncultured Shewanella sp.</i>		29	10	-
				<i>uncultured Thalassotalea sp.</i>		9	1	-
				<b>Photobacterium</b>		<b>404,774</b>	<b>150</b>	159
				—		198,025	136	-
				<i>Photobacterium damsela subsp. damsela</i>		203,839	98	-
				<i>Photobacterium swingsii</i>		2,878	71	-
				<i>Photobacterium gaetbulicola Gung47</i>		14	4	-
				<i>uncultured gamma proteobacterium</i>		11	1	-
				<i>Photobacterium rosenbergii</i>		5	1	-
				<i>Photobacterium damsela subsp. piscicida</i>		2	1	-
				<b>Aliivibrio</b>		<b>328,413</b>	<b>130</b>	159
				—		273,651	123	-
				<i>Aliivibrio fischeri</i>		54,762	108	-
				<b>Enterovibrio</b>		<b>29,895</b>	<b>77</b>	159

	—	29,812	74	-
	<i>Enterovibrio nigricans</i>	42	5	-
	<i>uncultured bacterium</i>	41	5	-
<b>Salinivibrio</b>	—	<b>55</b>	<b>9</b>	159
<b>Grimontia</b>	—	<b>29</b>	<b>7</b>	159
<b>uncultured</b>	—	<b>57</b>	<b>6</b>	159
	—	<b>15750</b>	<b>140</b>	159