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1 Temporal and spatial dynamics of *Vibrio* 2 *harveyi*: an environmental parameter 3 correlated investigation in a 4-metre-deep 4 aquaculture tank

5 A. Da Fonseca Ferreira, R. Roquigny, T. Grard and C. Le Bris
6 Univ. Littoral Côte d'Opale, UMRt 1158 BioEcoAgro, USC ANSES, INRAe, Univ. Artois, Univ. Lille, Univ. Picardie Jules
7 Verne, Univ. Liège, Junia, 62200 Boulogne-sur-Mer, France

8 Abstract

9 Nowadays, European sea bass (*Dicentrarchus labrax*) aquaculture knows a significant expansion.
10 Nevertheless, the aquaculture industry is plagued by several microbial diseases, including vibriosis. The
11 spatial and temporal dynamics of pathogenic *Vibrio harveyi* were studied on a European seabass farm in
12 northern France over seven months in 2022. Cylindrical concrete specimens were suspended in a water
13 column and water was pumped from different depths (top: 0.3 m deep, middle: 2.15 m deep, and bottom:
14 4 m deep), providing insights into biofilm and planktonic *V. harveyi* dynamics. The abundances of *V.*
15 *harveyi*, in biofilm and free-living forms, were positively correlated. Water parameters were monitored,
16 revealing seasonal fluctuations in temperature, pH, and salinity, with no significant differences observed
17 across the water column. Fish mortality peaks were recorded in July and August, coinciding with elevated
18 temperatures and emphasizing the impact of environmental factors on aquaculture health. Quantification
19 of *V. harveyi* by quantitative PCR revealed no significant differences between water top, middle and
20 bottom layers. *Vibrio harveyi* exhibited seasonality, with peak abundances observed in August, correlating
21 with temperature increases. Principal component analysis identified temperature as a primary driver, but
22 also additional parameters such as salinity and pH. vibriosis outbreaks occurred during the sampling
23 period, providing valuable insights on conditions before, during, and after the outbreak. This study
24 underscores the importance of understanding *V. harveyi* behaviour in aquaculture systems, particularly
25 in the context of global warming, for effective disease management and sustainable practices.

26 Keywords

27 Aquaculture, *Vibrio harveyi*, Biofilms, Planktonic, Environment, Spatio-temporal study

28 1. Introduction

29 Given the swift expansion of the global population and the sharp decrease in catches from marine
30 fisheries, the advancement of aquaculture has gained substantial attention as a means to guarantee
31 worldwide food supplies (Ahmed et al., 2019). The European seabass (*Dicentrarchus labrax*) stands out as
32 one of the most economically significant fish species harvested in Europe. Over recent decades, the
33 European seabass aquaculture sector has experienced robust growth. Overall production has reached
34 80,786 tonnes, amounting to a value of € 470 million in 2020 (EUMOFA, 2022). However, like many other
35 species, *D. labrax* is susceptible to various bacterial diseases, including pasteurellosis (*Photobacterium*
36 *damselae* subsp. *piscicida*), photobacteriosis (*Photobacterium damsela* subsp. *damsela*),
37 tenacibaculosis (*Tenacibaculum* spp.), and vibriosis (*Vibrio* spp.). These diseases pose significant threats
38 to the seabass aquaculture sector (Muniesa et al., 2020). They can not only cause mass mortality events,
39 but also have substantial economic consequences (ANSES, 2019; Novriadi, 2016).

40 Throughout the whole production chain, vibriosis stands out as the most significant threat to seabass. It
41 is characterised by various symptoms in seabass, including anorexia, erratic swimming behaviour,
42 exophthalmia, darkening of body colour, pale gills, haemorrhage, ulcers, and skin lesions, and can be fatal

43 (Austin and Zhang, 2006; Korun, 2008; Mohamad et al., 2019). It can be caused by various species of
44 *Vibrio*, such as *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio ordalii*, *Vibrio*
45 *parahaemolyticus*, and *Vibrio vulnificus* (Korun, 2008; Muniesa et al., 2020; Novriadi, 2016; Pujalte et al.,
46 2003). Furthermore, the range of *Vibriosis* spp. is expanding, posing an ongoing challenge in open-circuit
47 aquaculture systems supplied with water directly from the open sea (Baker-Austin et al., 2017).
48 Prophylactic measures, such as commercial vaccines, have been extensively developed and successfully
49 implemented to combat *Vibrio anguillarum* (Dadar et al., 2017). Now that the issue of *V. anguillarum* is
50 well managed, *V. harveyi* has recently emerged as a significant concern in seabass aquaculture (Abu Nor
51 et al., 2020; Islam et al., 2022; Vendramin et al., 2001).

52 *Vibrio harveyi* thrives in natural marine ecosystems. Its ability to act as a pathogen in a wide range of
53 cultivated hosts, such as crustaceans, molluscs, and fish is a concern notably for open-circuit aquaculture
54 farms (Austin and Zhang, 2006). Importantly, *V. harveyi* exhibits various states of existence, including free-
55 swimming and within biofilms attached to both living and non-living surfaces, or engaged in symbiotic and
56 host-pathogen interactions (Montánchez and Kaberdin, 2020). The formation of biofilms on tank walls
57 can significantly heighten the risk of vibriosis outbreaks. Besides, outbreaks of vibriosis in seabass farms
58 exhibit pronounced seasonality, occurring more frequently when water temperatures reach their
59 maximum (Pujalte et al., 2003). Increasing temperatures, particularly with global warming, may boost the
60 abundance and possibly the virulence of viable *V. harveyi* in the surrounding environment, consequently
61 increasing the presence of *V. harveyi* and its damages in tanks. Actually, the occurrence of vibriosis
62 episodes remains unpredictable despite high temperatures, as highlighted by Mougín et al. (2021). In their
63 study, conducted in a small tank (75 m³), no instances of vibriosis occurred during the 7-month sampling
64 campaign, despite the clear impact of temperature on *V. harveyi* concentrations. The study was
65 conducted in a 1.3-metre-deep tank, which is not deep enough to observe the heterogeneity in
66 environmental parameters and their potential impact on *V. harveyi* and vibriosis development. This
67 underscores the need for further investigations into the relationships between environmental
68 parameters, *V. harveyi* concentrations dynamics, and vibriosis outbreaks. Additionally, to gain a more
69 comprehensive understanding of these relationships, studies should expand to larger tanks, where
70 environmental heterogeneity is more likely, allowing for a more nuanced examination of repartition
71 within the water column. Importantly, biofilms add an additional layer of complexity to the understanding
72 of vibriosis development. In biofilms, interspecific interactions are facilitated, and previous research
73 conducted by Roquigny et al. (2021) has already highlighted distinctions in microbial communities and
74 their temporal shifts in an aquaculture tank which may interact with *V. harveyi*. In this context, a first
75 question arises whether the temporal and spatial dynamics of these two forms of *V. harveyi* — free-
76 swimming and within biofilms — are interconnected, and if one compartment of the tank acts as a
77 reservoir for each form. This uncertainty complicates the understanding of vibriosis onset, introducing
78 additional challenges in managing and mitigating the impact of *V. harveyi* in open sea environments. In
79 addition to the complexities posed by *V. harveyi*'s presence in various states, vibriosis development is not
80 solely governed by host-pathogen interactions. Rather, it constitutes a multifaceted tripartite
81 relationship, influenced by environmental biotic and abiotic factors (Maulvault et al., 2018). These factors,
82 such as fish density, water temperature, salinity and pH, may collectively play a significant role in shaping
83 the dynamics of vibriosis outbreaks.

84 Thus, it is essential to comprehend *V. harveyi*'s spatial and temporal distribution within aquaculture tanks,
85 as well as the environmental conditions that favour its proliferation and persistence. Therefore, through
86 a comprehensive investigation conducted from May to November 2022, encompassing sampling periods
87 before, during, and after episodes of vibriosis outbreaks, we sought to elucidate the spatial persistence
88 of *V. harveyi* in water and biofilms particularly during "heatwaves" or vibriosis outbreaks. This sampling
89 was done through both biofilm and water sampling, and environmental factors were measured alongside.
90 Besides, the conducted sampling was done at three different depths within the water column in a 4-metre
91 deep tank, to capture potential variations across vertical gradient. This study aimed to identify potential
92 environmental niches within these tanks where *V. harveyi* may thrive and persist, potentially leading to
93 vibriosis outbreaks. It could lay the groundwork necessary to effectively address its virulence and mitigate
94 the risk of vibriosis in aquaculture settings. Indeed, with escalating concerns over antibiotic resistance and

95 changing consumer preferences favouring sustainable practices, relying on antibiotic use is not a viable,
96 long-term solution. In this “One Health” context, aquaculture practitioners could solve this problem by
97 devising targeted antivirulence strategies that not only address the immediate challenges posed by *V.*
98 *harveyi*, but also align with the principles of sustainable and responsible aquaculture. The goal of this
99 research was to contribute to new strategies for effective management and regulation of vibriosis in
100 aquaculture facilities, based on informed preventive measures.

101 2. Material & methods

102 2.1 Sampling site and rearing conditions

103 From May to November 2022, a sampling campaign was conducted at Aquanord-Ichtus, an aquaculture
104 establishment situated in Gravelines (France), along the coast of the North Sea. This facility is strategically
105 located near a nuclear power plant, which serves as a source of warm water for the flow-through tank
106 systems via its reactor water cooling system. The facility benefits from thermoregulated water throughout
107 the year, except in summer when the facility faces challenges in cooling the water tank more than
108 environmental water.

109 The cubical aquaculture tank chosen is an outdoor facility with a volumetric capacity of approximately
110 975 cubic m, a height of 4 m, and a capacity of up to 68,000 sea bass. The average fish stock density within
111 the tank was 32 kg per cubic meter at the beginning of the campaign, and the average individual fish
112 weight was 517 g.

113 Fish are fed daily and oxygenation is carried out to maintain ideal dissolved oxygen (DO) levels. To
114 overcome the absence of available vaccines against *V. harveyi*, Aquanord-Ichtus has started to create self-
115 vaccines. This approach enables farmers to use oxytetracycline, an antibiotic, only in extreme cases when
116 vibriosis -induced mortalities are exceptionally high and not effectively managed by other zootechnical
117 measures. During vibriosis outbreaks and elevated fish mortality on the farm, the farmers administered
118 oxytetracycline treatment for 7–10 days, ensuring antibiotic coverage persists for about 4 weeks.

119 2.2 Sample collection and environmental parameters

120 To study the spatial and temporal dynamics of *V. harveyi* in biofilms, cylindrical concrete specimens (5 x
121 2.5 cm) constructed from the same material as the tank walls, were attached to a rope at three equidistant
122 depths from the surface of the tank (top: 0.3 m deep, middle: 2.15 m deep, and bottom: 4 m deep). At
123 each sampling time, five replicates of concrete were sampled at each depth. To study planktonic *V.*
124 *harveyi*, alongside the concrete samples, 1 L of water in five replicates was also collected at each depth
125 using a submersible pump. This process yielded 16 independent sampling times, and samples taken from
126 16 ropes at the wall of the tank. Sampling apparatus was installed when the tank was in an empty state,
127 and the initial sampling was done one day after impoundment of the tank when the fish were added.

128 Biofilm and planktonic samples were collected every three weeks. Throughout the sampling campaign,
129 environmental seawater temperatures were carefully monitored, based on data provided by the MAREL
130 Carnot buoy consulted from May to November 2022 (MAREL Carnot, 2022). This buoy supplied daily
131 insights into the environmental temperatures of water used to partially supply the tanks. When seawater
132 temperatures exceeded 19°C for two consecutive nights, sampling events were scheduled within a span
133 of 4–5 days to anticipate the onset of vibriosis episodes. Given the sustained temperatures above 20°C
134 from mid-July to the end of August, a weekly sampling regimen was adopted, amounting to 7 samples
135 (Supplementary Table 1). At each sampling time, abiotic parameters such as water temperature, pH,
136 salinity, turbidity, DO, and conductivity were assessed within the tank at each layer, using an HI-9892
137 multi-parameter probe (Hanna Instruments, Leighton Buzzard, United Kingdom). Throughout the
138 sampling campaign, the aquaculture facility supplied weekly data concerning biomass and mortality rates
139 for the studied rearing tank. A total of 240 water samples and 240 biofilm samples were collected during
140 the sampling campaign.

141 2.3 Bacterial isolation

142 The experimental protocol, established for a previous study in Aquanord-Ichthus (Mougin et al., 2020), was
143 replicated with minor adaptations. Briefly, we gathered five replicates of 1 L seawater samples from
144 aquaculture tanks at different depths and sampling times. These samples were promptly chilled on ice
145 during transportation to the laboratory and processed within a 3-hour window following the sampling.
146 Filtration involved the sequential use of 0.45 µm and 0.22 µm-pore-size nitrocellulose filters (Grosseron
147 SAS, Couëron, France), with the deployment of multiple filters when necessary to prevent clogging due to
148 sample aggregation. Following this, the filters were immersed in a solution consisting of 30 mL of Luria-
149 Bertani broth supplemented with 20 % NaCl (LBS) containing 20 % glycerol and stored at -80°C until further
150 analysis.

151 Concrete specimens were submerged at various depths: 0.3, 2.15, and 4 m. Post-collection, the specimens
152 were individually collected and preserved in sterile physiological water and put on ice during
153 transportation to the laboratory. They were immediately processed within a 2-hour timeframe upon
154 arrival. The biofilm collection protocol from concrete, following the Mougin et al. (2020) guidelines with
155 slight adaptations, used a systematic swabbing methodology after rinsing the blocks with sterile
156 physiological water, targeting a specific surface area of 2.34 cm². This process involved use of a sterile,
157 stainless steel jig and two consecutive sets of swabs (AB300250, Labomoderne, Gennevilliers, France).
158 Subsequently, the swabs were immersed in LBS enriched with 20 % glycerol and stored at -80°C until
159 further analysis.

160 To begin the analytical phases, all samples were thawed and subjected to a 3-min vortexing process. After
161 centrifugation at 8,000 x g for 10 min, the resulting cell pellet was resuspended in physiological water. For
162 subsequent steps, the samples were divided into two subsamples, with one of them (1,800 µL) to be used
163 for DNA extraction and *V. harveyi* quantification through real-time PCR, and the other (200 µL) kept for
164 another study.

165 2.4 Quantification of *V. harveyi* using real-time PCR

166 The 1,800 µL subsamples underwent a centrifugation step at 8,000 x g for 10 min. Subsequently, a DNeasy
167 PowerBiofilm kit (QIAGEN, Hilden, Germany) was used for total DNA extraction from the cell pellet,
168 adhering to the manufacturer's instructions. This method was chosen based on the procedures described
169 by Mougin et al. (2020). This involved the use of *V. harveyi*-specific primers designed to target the *mreb*
170 gene, which is present in *V. harveyi*'s genome as a single copy. The primers were synthesized by TIB
171 MOLBIOL Syntheselabor GmbH (Berlin, Germany). To establish standard curves for quantification, a series
172 of 10-fold dilutions was prepared using pure DNA from the collection strain *V. harveyi* LMG 4044. For each
173 individual assay, calibration curves were generated by plotting the mean threshold cycle (CT) values
174 derived from two replicate series against the logarithm of the respective dilution factors. To mitigate
175 potential interference from real-time PCR inhibitors, each extracted DNA template was analysed after
176 being diluted tenfold. Genome copy equivalent (GE) concentrations were calculated, taking the dilution
177 factor into consideration. All quantitative PCR reactions were conducted using a LightCycler® 480
178 instrument (Roche Diagnostics, Meylan, France). These assays included a positive control (*V. harveyi* LMG
179 4044 DNA), a negative control (*V. campbellii* CIP 70.67 DNA), and a no-template control (NTC) devoid of
180 DNA. To confirm the specificity of the fluorescent signals, melting curve analysis was performed. To ensure
181 the reliability of measurements, each extracted DNA template underwent two rounds of analysis.

182 2.5 Statistical analysis

183 Data normality was assessed using either the Shapiro–Wilk test or the Kruskal–Wallis test, depending on
184 the dataset distribution. Subsequently, the homogeneity of variances was evaluated using Bartlett's test.
185 To determine significant differences among variables across different layers, a one-way analysis of
186 variance (ANOVA) was conducted. Post-hoc comparisons were carried out using the least significant
187 difference (LSD) method with Bonferroni-adjusted *p*-values. In instances where the data did not meet the
188 assumption of normality, the Kruskal–Wallis test was employed as a non-parametric alternative for
189 comparing medians among different groups. Additionally, a principal component analysis (PCA) was
190 performed on environmental parameters (temperature, pH, salinity, DO, and turbidity), *V. harveyi* water

191 concentration, *V. harveyi* concrete concentration, and fish mortality. Before conducting PCA, the data
192 were standardised to ensure equal weighting of each variable. Relationships among variables were
193 explored using a correlation matrix, employing the Spearman method. Prior to this analysis, pairwise
194 linear relationships were assessed to ensure the absence of linearity. All statistical analyses, including
195 post-hoc procedures, were conducted using R version 4.3.2 and R Studio version 2021.09.0 (R Core Team,
196 2023; RStudio Team, 2020) with the “agricolae” (De Mendiburu and Yaseen, 2020), “factomineR” (Lê et
197 al., 2008), “factoextra” (Kassambara and Mundt, 2016), and “corrplot” (Wei and Simko, 2021) packages.
198 All graphics were drawn using the “ggplot2” packages (Wickham, 2016).

199 3. Results

200 3.1 Water quality and fish mortality

201 The investigation involved a comprehensive examination of water parameters in the top, middle, and
202 bottom layers over the course of May to November 2022. Additionally, fish mortality data were collected
203 and analysed. These data are summarised in Table 1. No significant differences were found across the
204 water column for each sampling time for the recorded parameters, indicating homogeneity of
205 environmental conditions in the tank. Nevertheless, some parameters showed variations throughout the
206 sampling period. For instance, the mean temperature values ranged from a minimum of 16.70°C in
207 November to a maximum of 22.06 °C in August, demonstrating seasonal fluctuation. pH values gradually
208 decreased from an average of 8.07 in May to 6.87 in November. Values also showed increasing acidity
209 with depth in the water column. DO concentrations remained constant. Fish mortality percentages were
210 higher in July and highest in August when peak temperature values were recorded. Fish mortality episodes
211 in May were likely associated with stress induced by transfer of fish into the tank.

212 3.2 Quantification of *V. harveyi*

213 Abundance of *V. harveyi* was determined using *mreb*-targeted real-time PCR across the different layers
214 for all samples, as shown in (Fig. 1). The qPCR method has a specified threshold of detection in GE for each
215 sample type: 2.24 GE.mL⁻¹ (0.35 log(GE.mL⁻¹)) for water samples and 501.19 GE.cm⁻² (2.70 log(GE.cm⁻²))
216 for concrete samples. Despite the potential presence of *V. harveyi*, any concentration below this threshold
217 was considered to be zero in the analysis (Supplementary Table 2). For a given sampling time, no
218 significant differences were found through the water column for each sample type.

219 In water samples, the presence of *V. harveyi* started to be detected from the beginning of the sampling
220 campaign in May, persisting consistently thereafter. A significant higher *V. harveyi* concentrations was
221 observed in August compared to other months ($p < 0.05$). Of note, *V. harveyi* abundance in the bottom
222 layer ranged from 0.52 to 3.36 log(GE.mL⁻¹) (Fig. 1), with a distinct peak in August when the temperature
223 of the water tank was at its highest. Moreover, abundances in the middle and top layers exhibited similar
224 profiles through the months, ranging from 0.60 to 2.43 log(GE.mL⁻¹) and from 0.48 to 2.89 log(GE.mL⁻¹)
225 respectively. Conversely, when oxytetracycline was used during episodes of mortality, a decrease in *V.*
226 *harveyi* concentrations was observed in water samples, allowing for concentration maintenance below
227 1.90 log(GE.mL⁻¹), followed by a gradual increase towards the end of the antibiotic’s predicted effect.

228 Many samples were found to be below the qPCR detection threshold for the concrete samples. The
229 estimated concentration of *V. harveyi* in biofilm samples did not exhibit a significant difference over time
230 and through the water column. *Vibrio harveyi* started to be detected in June and ceased by the end of
231 October for top and middle tank samples. Bottom samples were detected until the end of the sampling
232 campaign. Detected abundances in the bottom, middle and top layers of the tank ranged from 2.88 to
233 4.51 log(GE.cm⁻²), 3.02 to 4.60 log(GE.cm⁻²), and 2.98 to 4.93 log(GE.cm⁻²), respectively (Fig. 1). A rapid
234 increase in *V. harveyi* abundance was observed during high-temperature periods, particularly from August
235 to September, resulting in two peaks during these months associated with fish mortality in the tank (Table
236 1). Statistical analysis revealed significant variations in *V. harveyi* concentrations for all layers, with August
237 and September forming a cluster characterised by concentrations significantly higher than those observed
238 for the rest of the months ($p < 0.05$). Moreover, during these episodes of mortality, oxytetracycline was
239 used and a resulting decrease in *V. harveyi* abundance was observed, enabling concentration maintenance

240 to remain below 3.97 log(GE.cm⁻²). The abundance of *V. harveyi* gradually rose as the antibiotic's predicted
241 effect started to fade.

242 3.3 Influence of environmental factors

243 PCA (Fig. 2) was combined with a correlation matrix (Fig. 3) to reveal the interactions of the parameters
244 measured in the aquaculture tank. Both dimensions of the PCA, Dim.1 and Dim.2, captured 64.40% of the
245 data variance, explaining 42.08% and 22.32% of total variance, respectively. Temperature, *V. harveyi*
246 abundance in water ([Vh water]), and *V. harveyi* in biofilm form ([Vh concrete]), emerged as the primary
247 drivers shaping the Dim.1 variance landscape, while Dim.2 was predominantly influenced by DO and
248 turbidity. Strong positive correlations (0.88, $p < 0.001$) were found between *V. harveyi* abundance in water
249 and its abundance in biofilm within the tank, as highlighted in the correlation matrix, indicating that the
250 biofilm results align closely with those observed in the water. A strong positive correlation was observed
251 in the PCA between temperature and both *V. harveyi* abundance in water (0.891, $p < 0.001$) and in biofilm
252 (0.834, $p < 0.001$), but also, to a lesser extent, salinity (0.772, $p < 0.001$). Further confirming and extending
253 these findings, the correlation matrix highlighted significant positive correlations such as temperature
254 with salinity (0.84, $p < 0.001$), *V. harveyi* concentration in water (0.63, $p < 0.001$), concrete (0.55, $p <$
255 0.001), and fish mortality (0.50, $p < 0.001$). In contrast, pH exhibited a negative correlation with *V. harveyi*
256 abundance in water (-0.43, $p < 0.001$), indicating an inverse relationship. On the other hand, Dim.2
257 revealed significant correlations with DO (0.798, $p < 0.001$), pH (0.566, $p < 0.001$), fish mortality (0.496, p
258 < 0.001), and turbidity (-0.741, $p < 0.001$). Additionally, the correlation matrix revealed strong negative
259 correlations between DO and turbidity (-0.40, $p = 0.0054$), and positive correlation with fish mortality
260 (0.34, $p = 0.0196$).

261 4. Discussion

262 4.1 Temporal and spatial study of *V. harveyi* in aquaculture

263 Our investigation examined the temporal and spatial distribution of *V. harveyi* throughout a sampling
264 campaign spanning May to November 2022 on a farm in northern France. This farm has experienced
265 recurring vibriosis episodes attributed to *V. harveyi*. A previous study conducted in a "small tank" (75 m³,
266 1.3 m deep), revealed the consistent presence and persistence of *V. harveyi* in both planktonic form and
267 biofilms throughout the year (Mougin et al., 2021). This previous study was restricted by the absence of
268 vibriosis outbreaks during the campaign, but also by the small tank size, which prevented in-depth
269 exploration of variations in environmental conditions and their impact on *V. harveyi*. To better understand
270 the dynamics of *V. harveyi* through the water column, we implemented sampling campaign in a 4-metre-
271 deep tank at three different depths. We systematically monitored environmental parameters to find
272 potential explanations for the observed variations. Our study sets itself apart by adopting a
273 comprehensive sampling approach over an extended time frame that included the periods before, during,
274 and after two episodes of vibriosis. Additionally, during our sampling campaign, the two vibriosis
275 outbreaks forced the farmers to use antibiotics. Consequently, this study offers insights into antibiotic
276 effects on *V. harveyi*, considering both planktonic and biofilm forms. This campaign also sheds light on the
277 omnipresence of *V. harveyi* within the aquaculture tank. From the very onset, brought in with the initial
278 filling of the tank with water, *V. harveyi* demonstrated its adaptability, swiftly establishing its presence in
279 the tank environment. *Vibrio harveyi* was present and quantifiable during the whole sampling campaign
280 in planktonic form in the water, and during warmer months in biofilms. Its abundance in biofilms
281 potentially increased due to persistence once established on the tank wall, a finding in line with the
282 previous sampling campaign on the same aquaculture farm in 2018 (Mougin et al., 2021). A distinctive
283 characteristic of *Vibrio*, including *V. harveyi*, is their ability to adapt to changing conditions. This
284 adaptability is underscored by their capacity to transition from free-swimming cells to "swarmer cells"
285 within biofilms (Khan et al., 2020; McCarter, 1999). Furthermore, during the warmer months, it is known
286 that the abundance of other coexisting bacterial families within biofilms such as *Rhodobacteraceae*
287 decreases, providing an environment that enables the *Vibrionaceae* to thrive in the microbial community
288 structure (Bourne et al., 2004; Roquigny et al., 2021)

289 4.2 Abundance of *V. harveyi* in the tank

290 In comparison to Mougin et al. (2021) our study observed higher concentrations of *V. harveyi* in our water
291 tank. They found, actually, a maximum concentration of 9.51×10^1 GE.mL⁻¹ in rearing tanks, while a
292 maximum concentration of 2.54×10^3 GE.mL⁻¹ was found in our water tank. This contrast could be due to
293 differences in environmental conditions, but also due to different tank sizes and fish. In our study, which
294 aimed to investigate the water column, all measured abiotic parameters in the water column exhibited
295 clear stability, a characteristic likely shaped by the conditions of fish farming. These conditions, which
296 ensure efficient water circulation, additionally play a role in homogenising tank parameters overall. The
297 concentrations of *V. harveyi* reflected the same stability through the water column. Similar observations
298 regarding *Vibrio* spp. concentrations in the water column were found in a natural oyster farming site (Cruz
299 et al., 2020). The only significant difference observed by Cruz et al. (2020) appeared for some oysters
300 collected close to the seafloor where sediment accumulation was high. Even though our tank water
301 appeared to be homogeneous, organic matter accumulated at the floor of the tank. This phenomenon is
302 caused by fewer fish activities at the bottom, resulting in less associated movement. It can also be
303 attributed to the sedimentation and accumulation of nutrient-rich sediments consisting of food scraps
304 and fish faeces. Moreover, sediments are known to serve as a source of nutrients for *V. harveyi*, which
305 not only sediment themselves in the tank, but are also able to absorb dissolved organic matter (Neogi et
306 al., 2018). Sediments also provide an additional surface for colonisation, protecting the bacteria from
307 biotic and abiotic stresses, and enabling their persistence in the tank (Pianetti et al., 2004; Vezzulli et al.,
308 2002). This hypothesis regarding sediment dynamics and *V. harveyi* colonization could be tested by
309 conducting further sampling after emptying the tank, which would provide insights into the role of
310 sediments in *V. harveyi* dynamics in aquaculture settings.

311 4.3 Environmental factors influencing *V. harveyi* distribution

312 Even though *V. harveyi* concentrations were spatially homogeneous throughout the water column,
313 temporal variations were observed through the 7-month sampling campaign. Relationships between
314 bacterial concentrations and measured environmental parameters were investigated through PCA (Fig. 2)
315 and a correlation matrix (Fig. 3). Interestingly, temperature emerged as the foremost factor influencing
316 the abundance of *V. harveyi*, as abundance was consistently noted to be more favourable as temperatures
317 rose above 20 °C. Given that *V. harveyi* was introduced into the tank water from the sea, its persistence
318 within the tank is facilitated by favourable tank conditions, enabling it to form biofilms on the tank walls
319 and promoting its growth. Additionally, extensive studies have demonstrated the crucial influence of
320 temperature on the presence of *V. harveyi* in natural aquatic systems, displaying a clear seasonal cycle,
321 particularly during the summer when temperatures exceed 25 °C (Chimetto et al., 2008; Zhou et al., 2012).
322 This seasonality was also observed in our study, as *V. harveyi* exhibited higher abundance during the
323 warmest months, when temperatures reached up to 22 °C. However, it is important to note that vibriosis
324 does not necessarily occur when temperature reach 22 °C, suggesting that additional factors may
325 influence its occurrence. Additionally, the correlation matrix (Fig. 3) suggests that salinity and pH, which
326 are predicted to change with overall ocean temperatures (Gulev et al., 2021), also positively affected the
327 presence of *V. harveyi* in the aquaculture tank. These findings are consistent with prior researches on the
328 effects of environmental parameters on *Vibrio* species in natural and in aquaculture settings, where a
329 positive correlation between salinity and temperature with the frequency of detection of *Vibrio* species
330 has been reported (Abioye et al., 2021; Scro et al., 2022). DO concentration is reported to have a negative
331 correlation with temperature, given that increased water temperatures lead to decreased DO levels
332 (Pfeffer et al., 2003). However, in our case, DO levels were managed continuously by the farmers, which
333 explains the absence of any observable effect on the abundance of *V. harveyi*. A second negative
334 correlation was observed with pH values. Although *V. harveyi* is known to tolerate wide ranges of pH levels
335 (i.e., 5.5 to 9.0), the correlation matrix in our study shows that pH is negatively correlated with both *V.*
336 *harveyi* abundance in water and salinity (Prayitno and Latchford, 1995). This parameter cannot be fully
337 controlled in aquaculture, adding another challenge to vibriosis management in tanks. In addition, global
338 warming and ocean acidification may alter the microbial balance, favouring the presence of *Vibrio*, while
339 diminishing populations of other bacterial genera (Montánchez and Kaberdin, 2020). All these

340 observations highlight the complexity to identify the multiparameter ideal *Vibrio* environment; varying
341 based on geographic location and environmental conditions (Trinanes and Martinez-Urtaza, 2021).

342 4.4 Challenges and considerations in managing vibriosis outbreaks

343 The repeated occurrences and unpredictability of vibriosis outbreaks associated with *V. harveyi* present a
344 significant challenge to the seabass aquaculture sector, as highlighted by Vendramin et al. (2001) and
345 Firmino et al. (2019). The issue becomes particularly problematic in open-system aquaculture, since the
346 prevalence of this pathogen in fish closely aligns with its abundance in the surrounding environment (Kim
347 and Lee, 2017), but also because water quality cannot be fully controlled. Moreover, in the context of
348 climate change, there has been a temperature increase of about 0.88 °C in sea and ocean surface
349 temperatures over the last century (Gulev et al., 2021). This temperature rise is known to affect the
350 distribution and abundance of marine organisms (IPCC, 2022), including *Vibrio*, which has seen its
351 abundance increase sharply in the North Sea over the period 1958–2011 (Vezzulli et al., 2002). According
352 to the Intergovernmental Panel on Climate Change, the ocean is projected to continue warming, leading
353 to long-term impacts on ocean ecosystems and human societies (IPCC, 2022). Open aquaculture systems
354 may therefore need to address an increase in vibriosis episodes. This heightened risk could require
355 increased antibiotic use, posing risks to fish health, environmental integrity, and consumer safety. In
356 response to these challenges, it is imperative to develop specific and effective solutions. Antibiotics, as
357 suggested by our findings, fall short as a sustainable solution, since despite their use by farmers, the
358 concentrations of *V. harveyi* in water and biofilms quickly rebound. Within a three-month period, farmers
359 had to resort to antibiotics twice to avoid massive fish losses (Fig. 2). Given the escalating importance of
360 addressing the *Vibrio* challenge in response to global warming, it is crucial to adopt a comprehensive
361 approach to understand the virulence mechanisms used by *V. harveyi*. As an opportunistic pathogen, *V.*
362 *harveyi* thrives in warmer temperatures, requiring proactive measures in response to changing climate
363 conditions that could now favour its growth in previously cooler waters. Addressing the core issue involves
364 reducing bacterial virulence through antivirulence therapies (Clatworthy et al., 2007; D'Angelo et al.,
365 2018). Environmental effects impact not only to behaviour of the bacterium, but also to its area of
366 distribution, which is bound to evolve. For some geographic areas, including the one in the present study
367 and more generally temperate waters, such changes may pose challenges regarding *Vibrio* expansion
368 (Trinanes and Martinez-Urtaza, 2021). Moreover, they may even compromise host immunity, thereby
369 enhancing the bacterium's potential to increase mortality. Understanding these multifaceted interactions
370 becomes a keystone in formulating nuanced and effective strategies aimed at mitigating the impact of
371 potential vibriosis outbreaks.

372 5. Conclusion

373 In conclusion, this study highlighted the challenges of managing *V. harveyi* outbreaks in aquaculture
374 systems. The persistence of *V. harveyi* pose major challenges, particularly during warmer months. The
375 high adaptability of *Vibrio* and particularly *V. harveyi*, implies its considerable permissiveness, rendering
376 the task of attempting to control the entire environment to prevent vibriosis highly challenging, especially
377 in the context of global warming and ocean acidification (Montánchez and Kaberdin, 2020). Additionally,
378 significant correlations were found between *V. harveyi* concentrations and various environmental
379 parameters, highlighting the complex dynamics influencing its distribution. While temperature emerged
380 as a primary influencer, the absence of vibriosis outbreaks every summer suggests the influence of
381 additional parameters such as salinity and pH. Furthermore, in the context of escalating *Vibrio* abundance
382 linked to rising global ocean temperatures, concerns arise regarding the heightened pathogenic potential
383 of certain *Vibrio* strains, potentially leading to an increased incidence of diseases in the marine
384 environment. This poses a real challenge for open aquaculture systems which are specifically impacted by
385 these temperature increases as they are unable to regulate water temperature during the hottest months.
386 In navigating these intricate ecological challenges, the integration of a "One Health" framework becomes
387 imperative, emphasising the interconnectedness of animal, environmental, and human health (McEwen
388 and Collignon, 2018; Zinsstag et al., 2011). Understanding the virulence mechanisms of *V. harveyi* is crucial
389 for developing effective countermeasures and adaptive strategies that align with changing ecological
390 systems. This emphasizes the need to explore its virulence genes and their responses to environmental

391 factors like temperature, which are expected to evolve in the future. These insights are crucial for guiding
392 future research and developing strategies for disease management and sustainable aquaculture practices.

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544

545 *Figure 1: Biofilm concentrations of V. harveyi in log genome copy equivalents (GE).cm⁻² and V. harveyi concentrations in log*
 546 *GE.mL⁻¹ according to tank depth and time. The coloured lines correspond to the averages of the 5 replicates, which are*
 547 *represented by the shapes. Antibiotic treatments with oxytetracycline are indicated in dark green, and their period of effect is*
 548 *estimated in light green. The dashed line marks the qPCR detection threshold for V. harveyi quantification, delineating the limit*
 549 *above which presence is reliably detected.*

550 *Figure 2: Circle of correlation of the PCA for parameters including V. harveyi in water ([Vh water]), V. harveyi in biofilms ([Vh*
 551 *concrete]), dissolved oxygen, temperature, salinity, turbidity, pH, and fish mortality (Mortality). The contribution of each*
 552 *variable indicates its importance in explaining the overall variance in the dataset.*

553 *Figure 3: Correlation matrix of tank environmental parameters including V. harveyi concentrations in water ([Vh Water]), V.*
 554 *harveyi in biofilms ([Vh Concrete]), dissolved oxygen, temperature, salinity, turbidity, pH, and fish mortality (Mortality). The size*
 555 *of the circles corresponds to the strength of correlation, with larger circles indicating stronger correlations and small ones*
 556 *indicating weak correlations. Only statistically significant correlations ($p < 0.05$) between the parameters are shown.*

557 *Table 1: Mean values \pm standard deviations of parameters recorded from May to November 2022 in aquaculture facilities,*
 558 *categorised by the top, middle (mid), and bottom (bot) layers of the rearing tank. The 'n' signifies the number of sampling times*
 559 *averaged. For each sampling time, the average was calculated from 10 probe measurements.*

		Sampling month						
		May (n = 2)	June (n = 2)	July (n = 3)	August (n = 4)	September (n = 2)	October (n = 2)	November (n = 1)
Temperature (C°)	Top	18.29 \pm 0.77	19.06 \pm 0.30	20.89 \pm 0.28	22.06 \pm 0.58	20.69 \pm 0.03	17.82 \pm 0.36	16.71 \pm 0
	Mid	18.29 \pm 0.74	19.06 \pm 0.30	20.89 \pm 0.28	22.05 \pm 0.57	20.71 \pm 0.05	17.82 \pm 0.36	16.70 \pm 0
	Bot	18.29 \pm 0.74	19.06 \pm 0.30	20.89 \pm 0.28	22.06 \pm 0.57	20.71 \pm 0.04	17.82 \pm 0.36	16.70 \pm 0
pH	Top	8.07 \pm 0.71	7.53 \pm 0.16	7.37 \pm 0.22	7.29 \pm 0.04	7.39 \pm 0.06	7.17 \pm 0.19	7.06 \pm 0
	Mid	8.06 \pm 0.70	7.55 \pm 0.06	7.31 \pm 0.24	7.23 \pm 0.07	7.33 \pm 0.03	7.12 \pm 0.17	7.03 \pm 0
	Bot	7.95 \pm 0.72	7.51 \pm 0.02	7.25 \pm 0.23	7.18 \pm 0.07	7.25 \pm 0.08	7.02 \pm 0.16	6.87 \pm 0
Salinity (psu)	Top	33.83 \pm 0.68	34.75 \pm 0.16	35.14 \pm 0.16	35.07 \pm 0.16	34.97 \pm 0.11	34.09 \pm 0.05	33.08 \pm 0
	Mid	33.40 \pm 1.34	34.74 \pm 0.18	35.17 \pm 0.18	35.16 \pm 0.07	34.91 \pm 0.05	34.00 \pm 0.18	31.99 \pm 0
	Bot	33.45 \pm 1.34	34.63 \pm 0.27	35.12 \pm 0.15	35.18 \pm 0.10	34.83 \pm 0.20	33.81 \pm 0.30	32.67 \pm 0
Dissolved oxygen (mg.L ⁻¹)	Top	5.27 \pm 0.15	4.13 \pm 0.04	3.20 \pm 0.01	4.70 \pm 0.21	5.46 \pm 0.02	3.47 \pm 0.06	6.47 \pm 0.15
	Mid	5.45 \pm 0.03	5.19 \pm 0.02	3.39 \pm 0.01	3.63 \pm 0.02	5.83 \pm 0.09	3.46 \pm 0.04	3.64 \pm 0.26
	Bot	5.74 \pm 0.03	5.49 \pm 0.03	3.44 \pm 0.02	4.04 \pm 0.03	7.30 \pm 0.43	3.82 \pm 0.05	3.65 \pm 0.11
Turbidity (FNU)	Top	4.51 \pm 1.02	5.02 \pm 0.07	4.90 \pm 1.44	2.84 \pm 0.82	6.89 \pm 0.52	7.15 \pm 4.95	3.30 \pm 0
	Mid	4.26 \pm 1.32	3.40 \pm 1.45	4.17 \pm 0.75	3.86 \pm 0.83	4.64 \pm 1.65	5.56 \pm 2.36	6.67 \pm 0
	Bot	3.99 \pm 0.47	3.99 \pm 0.77	6.41 \pm 2.73	3.83 \pm 0.80	6.39 \pm 3.13	5.35 \pm 1.77	5.29 \pm 0

Fish Mortality (%)							
	1.26	0.98	1.31	4.57	0.90	0.06	0.01

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