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1 **Benthic foraminifera to assess ecological quality statuses: the case of salmon fish farming**

2

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21

22 **Keywords:** Fish aquaculture – English Channel – Environmental monitoring – Benthic  
23 foraminifera – Biotic index – Macrofauna

24

25 **Abstract**

26 The “Rade de Cherbourg” (RdC, Cotentin) hosts the only marine salmon fish farm along the  
27 French coasts. High hydrodynamic regime would limit, there, organic matter (OM)  
28 accumulation directly under the cages, and enhance the transport of OM in the surrounding  
29 of the cages. This study was aiming at (1) monitoring the impact of a salmon fish farm on  
30 ecological quality statuses (EcoQs) of the RdC based on a benthic foraminiferal biotic index,  
31 (2) comparing EcoQs assessment results between foraminifera and macrofauna, and (3) *in*  
32 *fine* assessing the potential for benthic foraminifera to become an alternate biological  
33 quality element. In 2014 and 2015, bottom sediments of the RdC were sampled at 13  
34 stations under and outside the farm for sedimentary (grain size and OM), and living  
35 foraminiferal and macrofaunal analyses. For benthic foraminifera,  $Exp(H'_{bc})$  was used to  
36 determine EcoQs, while  $H'$ , AMBI and BO2A indices were used for benthic macrofauna.  
37 Rank-frequency distributions (RFDs) were calculated for both groups. Ecological quality  
38 statuses based on foraminifera and macrofauna indicated a moderate degradation of the  
39 environmental conditions, shifting from excellent outside the farm to poor under the cages  
40 for foraminifera and from excellent to moderate for macrofauna. This study showed that  
41 benthic foraminifera are as reliable as macrofauna to assess EcoQs in the RdC. It offers  
42 interesting perspectives to monitor the health of marine systems based on benthic  
43 foraminifera. Furthermore, results obtained with RFDs suggested that this approach should  
44 be considered in the assessment of the good environmental status within the European  
45 marine strategic framework directive. Finally, diversity proved to be efficient in monitoring

46 the health of the RdC, suggesting that it should not be set aside for the benefit of sensitivity-

47 based indices.

48

## 49 **1. Introduction**

50

51           During the last 30 years, aquaculture in marine waters has greatly increased partly  
52 driven by the need for greater self-sufficiency in marine food production (Holmer, 2010).  
53 However, it is now widely acknowledged that activities related to aquaculture cause  
54 environmental disturbances (Bouchet and Sauriau, 2008; Carvalho et al., 2006; Chamberlain  
55 et al., 2001). Numerous studies have demonstrated that aquaculture degrades both  
56 sedimentary characteristics and benthic communities (Bouchet and Sauriau, 2008;  
57 Karakassis et al., 2002; La Rosa et al., 2004; Mazzola et al., 2000; Dauvin et al., 2020), which  
58 ultimately leads to decreased ecological quality statuses (Bouchet and Sauriau, 2008; Muxika  
59 et al., 2005). Previous studies show that the impacts of fish farms are essentially localised  
60 and depend mainly on aquaculture and environmental factors such as fish density, start date  
61 of activities, water depth, initial sea bottom site characteristics and hydrodynamic regime  
62 (Black, 2001; Karakassis et al., 2002; Yokoyama et al., 2006; Dauvin et al., 2020).

63           The Rade de Cherbourg (RdC), the second largest artificial roadstead in the world, is  
64 located on the north coast of the Cotentin Peninsula (Normandy, France) and hosts the only  
65 open marine water French salmon farm since the beginning of 1990s. Sediments directly  
66 below the cages are characterized by a moderated and localized increase in mud, organic  
67 carbon and nitrogen content (Kempf et al., 2002; Baux et al., 2017; Dauvin et al., 2020). Both  
68 the high hydrodynamic regime in the RdC and the acceleration of currents under the cages  
69 (Poizot et al., 2016) may often enhance the dispersion of excess organic waste from the  
70 salmon fish farm and limit long term accumulation of organic matter (OM). Hence, there are  
71 most likely a shift between periods of accumulation and then dispersion of the sediment  
72 under the cages. Accumulated OM under salmon farms may leads to anoxia in the sediment

73 (Nickell et al., 2003), hence generate further constraints on the related benthic  
74 communities. So far, only two studies described the soft bottom macrobenthic communities  
75 and the associated habitats in the RdC (Andres et al., 2020; Baux et al., 2017) and no  
76 information is available on meiobenthic communities. In addition, the salmon cages in the  
77 RdC have been shown to induce high to moderate Ecological Quality status (EcoQs), but only  
78 on the basis of a macrobenthic community assessment (Dauvin et al., 2020). There is  
79 consequently a need to monitor other components of the benthic ecosystem, an absolute  
80 prerequisite to assess the health status of the RdC in general, and in relation to salmon  
81 farming in particular.

82         There is now a plethora of biological quality elements (BQE) used to assess the health  
83 of benthic ecosystem — *sensu stricto* EcoQs — for instance, benthic macrofauna (Borja et  
84 al., 2000), fish (Coates et al., 2007), seagrass (Krause-Jensen et al., 2005), macro-algae (Ar  
85 Gall et al., 2016), and more recently, benthic foraminifera (Bouchet et al., 2012). Note that  
86 over the last 20 years, benthic macrofauna has been by far the most widely used BQE to  
87 assess EcoQs (Birk et al., 2012; Borja et al., 2000; Bouchet and Sauriau, 2008; Dauvin, 2018;  
88 Dauvin et al., 2007; Pinto et al., 2009; Rombouts et al., 2013). Recent developments in  
89 benthic foraminiferal biotic indices (Bouchet et al., 2012, Alve et al. 2016, Dimiza et al. 2016)  
90 provide, however, further opportunities for the development and implementation of this  
91 meiobenthic group as an acknowledged biological quality element within marine legislations  
92 for EcoQs assessment.

93         Benthic foraminifera have increasingly been acknowledged as indicators of human-  
94 induced stresses (e.g. Alve, 1995; Francescangeli et al., 2016; Polovodova Asteman et al.,  
95 2015), such as oil spills (Morvan et al., 2004), heavy metals (Armynot du Châtelet et al.,  
96 2004), urban sewage (Melis et al., 2016), and aquaculture (Bouchet et al., 2007; Debenay et

97 al., 2015; Vidovic et al., 2014). Specifically, fish farms induce clear shifts in the community  
98 structure of benthic foraminifera (Pochon et al., 2015), promote tolerant species (Angel et  
99 al., 2000) and lead to moderate to poor EcoQs (Bouchet et al., 2018a). In Norwegian fjords,  
100 benthic foraminiferal communities significantly correlate with benthic macrofauna  
101 communities, indicating that foraminifera can also be considered as good indicators of  
102 environmental conditions (Bouchet et al., 2018b). Foraminiferal indices based either on  
103 diversity (Alve et al., 2009; Bouchet et al., 2013, 2012) and on the sensitivity of species to  
104 organic pollution (Alve et al., 2016; Barras et al., 2014; Dimiza et al., 2016; Jorissen et al.,  
105 2018), have been designed and successfully applied to assess EcoQs of benthic habitats (Alve  
106 et al., 2019; Bouchet et al., 2018a, 2012; Damak et al., 2020; Dijkstra et al., 2017; Dolven et  
107 al., 2013; El Kateb et al., 2020; Francescangeli et al., 2016; Melis et al., 2016). Alve et al.  
108 (2019) further demonstrated that foraminifera and macrofauna have similar indicator  
109 efficiency by applying multimetric index based on foraminifera (NQIf) as an alternative,  
110 which is an adaptation of the Norwegian Quality Index (NQI), an internationally  
111 intercalibrated macrofauna index. Based on their results, Alve et al. (2019) recommended  
112 the inclusion of foraminifera as Biological Quality Element within the European Water  
113 Framework Directive's guidelines (WFD). In a study of Italian transitional waters, benthic  
114 foraminifera have further been shown to be more accurate than benthic macrofauna to  
115 assess EcoQs (Bouchet et al., 2018a). Considering the difficulties to assess EcoQs in naturally  
116 stressed ecosystems (Dauvin, 2007; Dauvin and Ruellet, 2009; Elliott and Quintino, 2007),  
117 foraminifera may be a relevant alternative to macrofauna (Hess et al., in press). Because of  
118 their potential to reconstruct palaeo-environments (Alve, 1991; Alve et al., 2009; Hayward et  
119 al., 2004), an unique feature compared to benthic macrofauna (except for molluscs; Poirier  
120 et al., 2009), foraminifera are also good candidates to establish objective and reliable

121 reference conditions (Dolven et al., 2013; Francescangeli et al., 2016). In short, benthic  
122 foraminifera may be a relevant complement to benthic macrofauna to assess EcoQs. These  
123 two groups exhibit similar features. Both are benthic and sedentary organisms, their  
124 distribution patterns depending directly on the environmental conditions. Furthermore,  
125 foraminiferal and macrofaunal community compositions in SE Norwegian fjords were  
126 significantly correlated suggesting that benthic foraminiferal distribution patterns mirror  
127 those of benthic macrofauna (Bouchet et al., 2018b). Intercalibration of benthic  
128 foraminiferal and macrofaunal biotic indices further confirm the complementarity of these  
129 two groups (Alve et al., 2019). Hence, this study was designed to discuss the relative  
130 strength of the classical macrofauna tool and the “newcomers” benthic foraminifera one to  
131 assess ecological quality statuses in the RdC in the context of salmon fish farming.

132 In this context, the present study aims at (1) monitoring the impact of a salmon fish  
133 farm on ecological quality statuses (EcoQs) of the Rade of Cherbourg based on a benthic  
134 foraminiferal biotic index, (2) comparing EcoQs assessment results between benthic  
135 foraminifera and benthic macrofauna, and (3) assessing the potential for benthic  
136 foraminifera to become an alternate biological quality element.

137

## 138 **2. Materials and Methods**

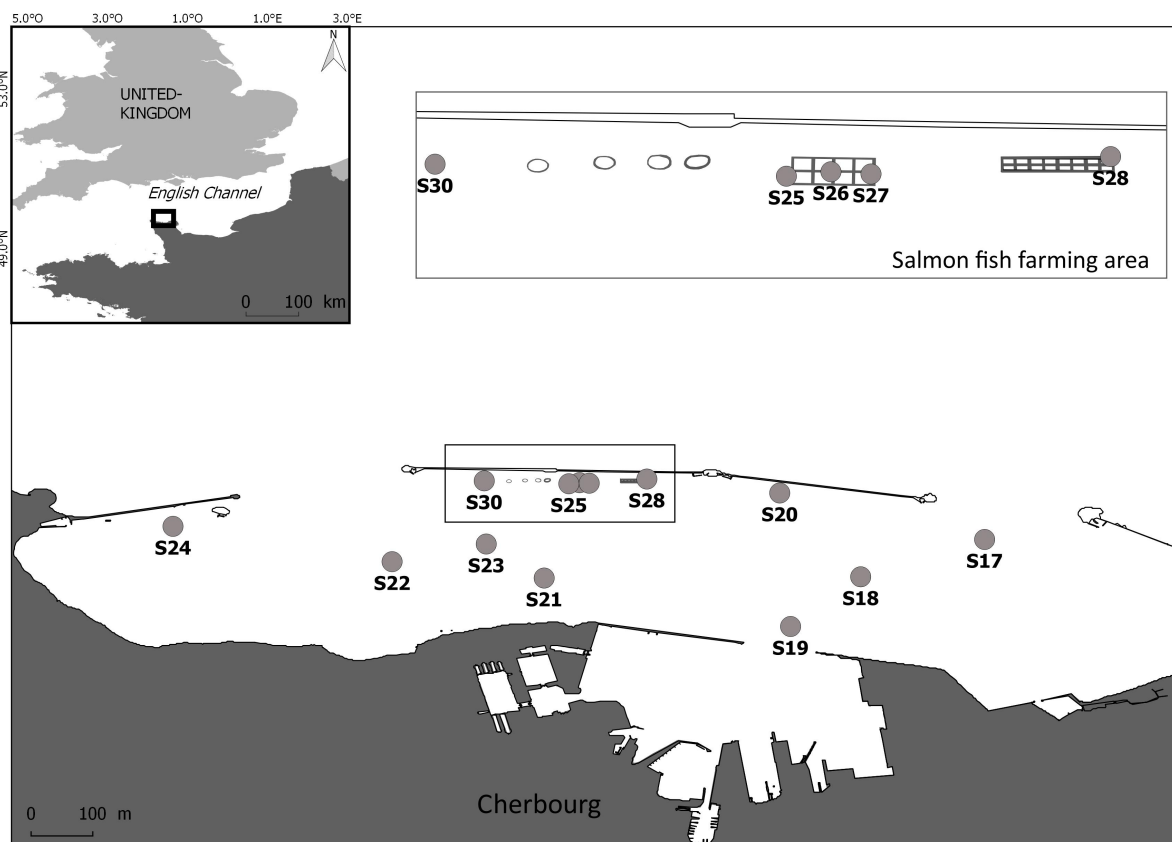
### 139 **2.1 Sedimentological, foraminiferal and macrofaunal sampling**

140

141 For the present study, thirteen stations (Fig. 1) were sampled in June 2015 in the RdC  
142 by scuba-diving using a push corer (diameter: 9.8 cm, layer: 0-1 cm, n = 3). Stations from S17  
143 to S24 were away the salmon fish farm in the RdC, while stations from S25 to S28 and S30  
144 were directly under the salmon cages (Fig. 1). The geographical positions of the stations



145 were determined using a RTK Global Position System (GPS ASHTECH Promark 120, accuracy  
146 better than 10cm). The study stations were visited for benthic macrofauna sampling in  
147 February 2014 at stations S17 to S22 and in February 2015 at stations S23 to S28 and S30.  
148 The sampling strategy used for the study of the macrobenthic communities is described in  
149 detail in Baux et al. (2017). Benthic macrofauna raw data are published in Baux et al. (2017)  
150 and Andres et al. (2020).  
151



152  
153 Figure 1: Sampling sites in the Rade de Cherbourg for benthic foraminifera (this study) and  
154 benthic macrofauna (Baux et al. 2017), sites are labelled following Baux et al. (2017).  
155

156 On board, for the present study, each replicate sample was split into two aliquots  
157 stored in polyethylene jars. The first one was used for sedimentological analyses (see “2.2

158 Fine fraction analysis” and “2.3 Organic matter analysis”), and the second for the assessment  
159 of the benthic foraminiferal assemblages (see “2.4 Foraminiferal analysis”). Sediment  
160 samples for the study of benthic foraminifera were stained with buffered rose Bengal dye (2  
161 g of rose Bengal in 1,000 ml of ethyl alcohol) to distinguish living from dead specimens (see  
162 Schönfeld et al., 2012 for the methodology). A comprehensive description of the  
163 environmental conditions characterizing each sampling station is available in the literature  
164 (Andres et al., 2020; Baux et al., 2017; Dauvin et al., 2020).

165

## 166 **2.2 Fine fraction analysis**

167

168 A total of 13 superficial sediment samples were collected by divers under the cages,  
169 and using a van Veen grab for the area located outside the farm. In both cases, great care  
170 was observed to sample only the first few centimeters. In the laboratory, samples were  
171 washed twice with distilled water to remove salts. Each sample was then dried at 30 °C. Wet  
172 sieving allowed separating the fine fraction <63 µm and the coarse one >63 µm. Each of  
173 them was oven-dried at 30 °C and finally weighed. Fine fraction percentages were then  
174 calculated.

175

## 176 **2.3 Organic matter analysis**

177

178 Before geochemical analysis, the fine fraction <63 µm was crushed and homogenized  
179 for each sample. Total organic carbon (TOC) contents were measured by combustion in a  
180 LECO CS 300. Three replicates of dried and homogenized sediment (50 mg) were analysed  
181 per station. Samples were heated to 1,600 °C and the amount of CO<sub>2</sub> was measured by

182 infrared absorption. For the analysis of Total Organic Carbon (TOC), sediment samples were  
183 acidified by HCl (12.5%) to remove carbonates. All inorganic carbon was assumed to be in  
184 the form of calcium carbonate. Quality control was maintained by measuring LECO certified  
185 reference materials (see further details on the method used for organic matter analysis in  
186 Baux et al., 2019).

187

## 188 **2.4 Foraminiferal analysis**

189

190 All samples were dried at 50°C and weighed. They were then gently washed with tap  
191 water through a 63 µm sieve to remove clay, silt and any excess dye and the residual fraction  
192 was re-dried at 50°C and weighed again to determine the mud fraction. Quantitative analysis  
193 of benthic foraminifera was performed on the fraction >63 µm. According to Murray and  
194 Bowser (2000), only specimens with dense, brightly red-stained protoplasm were considered  
195 as alive. When possible, three hundred stained specimens per sample were picked and  
196 identified, following the generic classifications of Loeblich and Tappan (1988).

197

## 198 **2.5 Data analyses**

### 199 *2.5.1 Rank-frequency diagram*

200

201 The structure of benthic foraminiferal and macrofaunal communities has been  
202 assessed using the rank-frequency relationship

$$203 \quad f_s = f(r + \phi)^{-\alpha} \quad (1)$$

204 where  $f_s$  is the frequency (i.e. relative abundance) of a species of rank  $r$  (the most abundant  
205 species has a rank  $r= 1$ , the second more abundant a rank  $r= 2$ , and so on, and the less

206 abundant species has a rank  $r = N$ , where  $N$  is the number of species found in a sample),  $f$  is a  
207 pre-factor based on the overall abundance of a community, and  $\alpha$  and  $\phi$  fitting parameter  
208 describing the diversity and evenness of a given community. Rank-frequency diagrams  
209 consist of frequencies of species plotted against their respective ranks organized in  
210 decreasing order, and with both axes in logarithmic scale (see examples in Fig. 2).

211 Specifically, when  $\phi = 0$  (i.e. Eq. (1) writes as  $f_s = fr^{-\alpha}$ ) a low value of  $\alpha$  means a slow  
212 decrease in species abundance (that is, a more even distribution of individuals among  
213 species), and a high value of  $\alpha$  means a rapid decrease of species abundance (that is, a more  
214 heterogeneous distribution). The former and the latter give less and more vertical rank-  
215 frequency distributions (RFDs), hence respectively high diversity/evenness and low  
216 diversity/evenness. On the other hand,  $\phi \neq 0$  describes the diversity and evenness of the  
217 most abundant species. Specifically, a positive value of  $\phi$  results in a greater evenness  
218 among the most frequent species, hence a higher diversity. Alternatively, a negative  $\phi$   
219 describes a community marked by the dominance of a few (even one) species and provides a  
220 low diversity index and a low evenness. The parameters  $\alpha$  and  $\phi$  were estimated for each  
221 sample using a nonlinear least-squares Levenberg–Marquardt algorithm, and were chosen  
222 as the values that respectively maximized and minimized the coefficient of determination  $r^2$   
223 and the sum of the squared residuals between empirical data and Eq. (1) (Seuront, 2013).

224

### 225 *2.5.2 Ecological Quality Statuses calculation*

226

227 Following the methodology proposed by Bouchet et al. (2012), EcoQs were  
228 determined with the diversity index  $\text{Exp}(H'_{bc})$  based on benthic foraminifera (Table 1).

229 Based on benthic macrofauna raw data published in Baux et al. (2017), the following  
 230 indices were calculated to assess EcoQs (Table 1): the diversity-based index Shannon  
 231 (Vincent et al. 2002) and the sensitivity-based indices BO2A (Dauvin and Ruellet, 2009) and  
 232 AMBI (Borja et al. 2000). The former BO2A index is based on the ratio between the  
 233 frequency of the Opportunistic Annelids and the frequency of the sensitive Amphipod  
 234 species (see review in Dauvin, 2018). The latter AMBI index is based on the classification of  
 235 species (or groups of species) into five ecological groups representing specific sensitivity  
 236 responses to an increasing gradient of Organic Matter (OM). The EG-I corresponds to taxa  
 237 sensitive to OM present in unpolluted conditions, EG-II to taxa indifferent to OM  
 238 enrichment, EG-III to taxa tolerant of excess OM enrichment, EG-IV, to second-order  
 239 opportunistic species present in high OM level, and EG-V to first-order opportunistic species,  
 240 able to resist to strong disturbance and excess in OM (Glémarec and Hily, 1981; Borja et al.,  
 241 2000).

242

EcoQS and associated colour code	Bad	Poor	Moderate	Good	High
Foraminifera - $\text{Exp}(H'_{bc})$	<5	5-10	10-15	15-20	>20
Macrofauna – $H'$	<1	1-2	2-3	3-4	>4
Macrofauna – BO2A	>0.25512	0.19885- 0.25512	0.13003- 0.19884	0.02453- 0.13002	<0.02453
Macrofauna - AMBI	>5.5	4.3-5.5	3.3-4.3	1.2-3.3	<1.2

243 Table 1. Criteria for determining EcoQS according to  $\text{Exp}(H'_{bc})$  – Bouchet et al. (2012),  $H'$  –  
 244 Vincent et al. (2002), BO2A – Dauvin (2018) – and AMBI – Borja et al. (2000).

245

246

### 2.5.3 Statistical analysis

247

248 Student t-test for unpaired data was performed to evaluate the null hypothesis that  
249 the percentage of fine fraction, TOC content and values of biotic indices did not differ  
250 between the two sampling areas (*i.e.* outside the farm vs. under the salmon cages).  
251 Correlations between the different biotic indices were calculated. Slope of RFDs'  $\alpha$  and  $\phi$   
252 correlations for foraminifera and macrofauna inside and outside the salmon farm were  
253 compared using ANCOVA analysis. Student t-test for unpaired data was run with the Past  
254 software version 3.24 (Hammer et al., 2001). Correlations were performed using the  
255 statistical language R version 3.4.1 (R Core Team, 2017) with the package *corrplot* version  
256 0.84.

257

### 258 3. Results

259

#### 260 3.1 Environmental parameters

261

Stations		Sediment		Biotic Indices			
		<63 (%)	TOC (%)	Foraminifera	Macrofauna		
				Exp(H' <sub>bc</sub> )	H'	BO2A	AMBI
Outside the farm	S17	5	0.13	11	2.1	0.080	2.2
	S18	16	0.40	8	3.1	0.010	2.4
	S19	52	0.80	18	2.5	0.030	2.2
	S20	42	0.83	22	4.5	0.020	0.9
	S21	43	0.91	18	2.3	0.070	2.1
	S22	20	0.40	20	1.9	0.010	2.4
	S23	19	0.64	17	2.3	0.010	2.4
	S24	64	1.67	15	2.3	0.070	2.4
Salmon farm	S25	18	0.49	8	2.1	0.050	3.0
	S26	14	0.33	11	2.8	0.170	4.0
	S27	28	0.50	8	2.9	0.150	3.7
	S28	36	0.60	10	2.7	0.070	3.0

	S30	11	0.33	14	1.8	0.010	2.6
--	-----	----	------	----	-----	-------	-----

262 Table 2: Grain size (fraction <63  $\mu\text{m}$ ), total organic carbon content (%) and biotic indices  
 263 values at sampling stations.

264

265 Results for the fine fraction (<63 $\mu\text{m}$ ) and total organic carbon (TOC) percentages  
 266 were summarized in the Table 2. The average percentage of the fine fraction represented  
 267 33 $\pm$ 7% outside the farm and 21 $\pm$ 5% under the salmon cages. There was no difference  
 268 between the two areas (t-test,  $p > 0.05$ ). Total organic carbon average contents in the  
 269 sediment remained low in the RdC, 0.72 $\pm$ 0.16% and 0.45 $\pm$ 0.05%, respectively outside the  
 270 farm and under the cages. There was no difference between the two areas (t-test,  $p > 0.05$ ).

271

### 272 3.2 Foraminiferal main species

273

274 Stations S19 to S24 are by Hyalin and Porcellaneous species, with varying proportions  
 275 of agglutinated species. The species *Discorbis vilardeboanus*, *Bolivina pseudoplicata*, *Bolivina*  
 276 *variabilis*, *Quinqueloculina stelligera*, and to a lesser extent *Miliolinella subrotunda*,  
 277 *Textularia truncata*, *Triloculina oblonga* and *Criboelphidium magellanicum*, characterised  
 278 the stations outside the salmon farm.

279 Under the cages of the fish farm, mostly *Bolivina variabilis* and *Quinqueloculina*  
 280 *stelligera* remained with relative abundances about 10-20% and 40%, respectively. Hyalin  
 281 and porcellaneous largely dominated the foraminiferal assemblages.

282 At stations S17 and S18, in the channel connecting the RdC to the open sea,  
 283 porcellaneous foraminifera over-dominated the assemblages with up to 70%, specifically

284 *Quinqueloculina stelligera*, *Miliolinella subrotunda* and *Triloculina oblunga* were the most  
285 abundant species.



		Outside the farm								Salmon farm				
		S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S30
Agglutinated	<i>Remaneica helgolandica</i>	0.0	0.0	0.0	0.0	0.0	<u>7.5</u>	3.7	0.2	1.0	0.0	0.0	0.0	0.8
	<i>Textularia truncata</i>	0.0	0.0	0.0	0.0	0.5	<u>6.8</u>	<u>6.9</u>	0.0	0.0	0.0	0.0	0.0	0.0
Porcellaneous	<i>Adelosina bicornis</i>	2.0	2.6	1.4	0.4	<u>6.9</u>	1.9	3.7	0.7	0.0	3.4	0.8	1.4	2.4
	<i>Adelosina dubia</i>	3.9	1.3	1.8	3.5	4.8	0.4	2.1	1.8	0.0	<u>5.1</u>	0.8	4.1	2.4
	<i>Miliolinella subrotunda</i>	<u>18.6</u>	1.3	0.0	<u>5.1</u>	0.0	3.0	0.5	0.9	0.0	<u>8.5</u>	0.0	<u>5.9</u>	0.0
	<i>Quinquelocina stelligera</i>	<u>30.4</u>	<u>50.7</u>	<u>12.6</u>	<u>7.1</u>	<u>22.9</u>	<u>7.1</u>	<u>17.6</u>	<u>20.5</u>	<u>38.0</u>	<u>44.9</u>	<u>43.7</u>	<u>40.0</u>	<u>22.6</u>
	<i>Quinqueloculina</i> sp.	0.0	2.6	0.0	0.0	0.5	0.0	0.0	0.0	<u>27.8</u>	0.8	0.0	0.9	0.0
	<i>Triloculina oblonga</i>	<u>9.8</u>	2.0	2.3	<u>5.5</u>	3.2	4.5	0.0	3.0	1.5	0.0	0.8	2.3	<u>6.5</u>
	<i>Triloculina triloculina</i>	4.9	<u>7.9</u>	0.5	0.0	4.8	1.1	1.1	0.3	0.0	2.5	2.4	<u>6.4</u>	0.8
Hyalin	<i>Bolivina pseudoplicata</i>	1.0	4.6	<u>16.7</u>	<u>9.8</u>	<u>14.4</u>	<u>12.8</u>	4.8	<u>10.2</u>	<u>5.4</u>	<u>3.4</u>	<u>7.9</u>	2.3	<u>8.1</u>
	<i>Bolivina variabilis</i>	2.9	<u>10.5</u>	<u>21.6</u>	<u>16.9</u>	<u>14.9</u>	<u>7.9</u>	<u>11.2</u>	<u>20.5</u>	<u>9.3</u>	<u>11.0</u>	<u>23.0</u>	<u>13.2</u>	<u>25.8</u>
	<i>Bulimina elegans</i>	2.0	1.3	<u>6.3</u>	3.5	0.5	1.5	0.0	1.5	0.0	1.7	0.8	0.9	1.6
	<i>Cribroelphidium gunteri</i>	0.0	0.0	0.9	2.4	0.0	0.0	0.0	<u>6.2</u>	0.0	0.0	0.0	0.0	0.0
	<i>Cribroelphidium magellanicum</i>	3.9	4.6	4.1	<u>7.1</u>	1.6	4.9	<u>8.0</u>	2.8	3.4	1.7	0.0	<u>6.8</u>	4.8
	<i>Discorbis vilardeboanus</i>	0.0	0.0	1.8	2.4	4.3	<u>14.7</u>	<u>20.2</u>	0.7	1.5	0.8	2.4	0.0	4.0
	<i>Hopkinsina atlantica</i>	0.0	0.7	3.6	<u>6.3</u>	2.1	0.0	0.0	<u>10.3</u>	0.0	0.8	0.8	0.5	4.8
	<i>Nonionella</i> sp.1	<u>5.9</u>	0.7	<u>6.8</u>	1.2	0.0	2.6	0.0	0.7	0.0	0.8	0.0	0.0	0.8
Agglutinated	2.0	0.0	1.4	8.6	4.3	21.4	17.0	1.8	4.4	2.5	2.4	2.7	3.2	
Porcellaneous	76.5	73.0	19.8	22.4	47.3	18.4	29.3	30.3	67.8	68.6	51.6	64.5	39.5	
Hyalin	21.6	27.0	78.8	69.0	48.4	60.2	53.7	67.8	27.8	28.8	46.0	32.7	57.3	

287 Table 3: Relative abundances of the main foraminiferal species (&gt;5%).

288

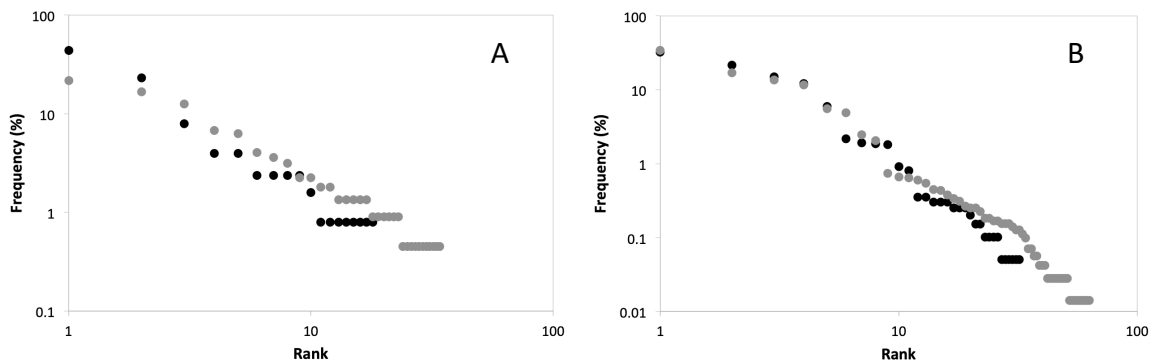
289

### 3.3 Rank-frequency diagrams

290

291 The parameters  $\alpha$  and  $\phi$  allowed for a synthetically analysis of the rank-frequency  
292 distributions (Fig. 2).

293



294

295 Figure 2: Typical examples of rank-frequency distributions of benthic foraminifera (A) and  
296 macrofauna (B) in the Rade de Cherbourg outside the farm (grey dots, station S19) and in  
297 the salmon farm (black dots, station S27).

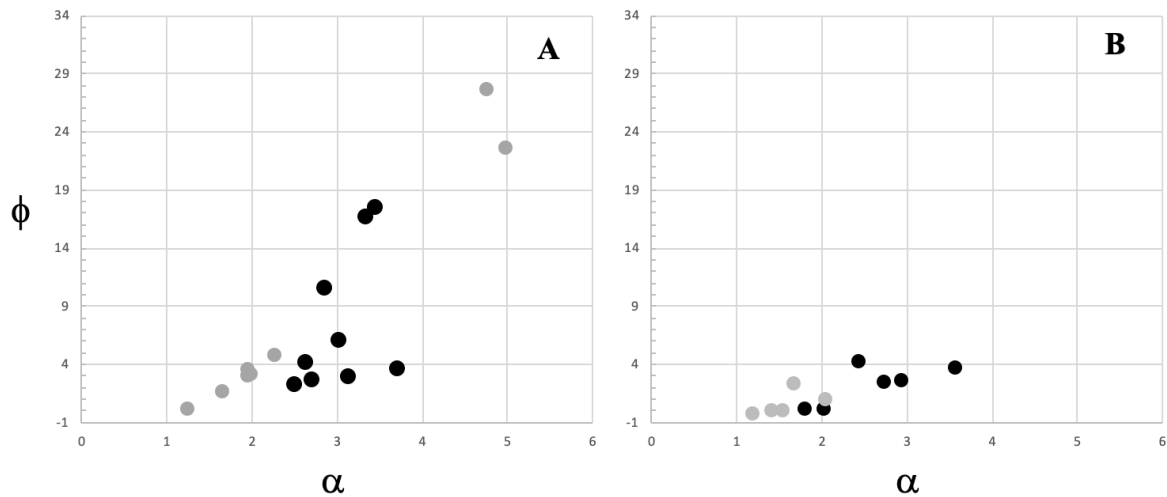
298

299 For foraminiferal assemblages, the parameters  $\alpha$  and  $\phi$  respectively ranged between  
300 1.82 and 3.57 and between 0 and 4.2 outside the farm, and between -0.3 and 2.3 and  
301 between 1.19 and 2.04 in the salmon farm (Fig. 3). For macrofaunal assemblages, the  
302 parameters  $\alpha$  and  $\phi$  respectively, ranged between 2.5 and 3.7 and between 2.2 and 17.5  
303 outside the farm, and between 1.25 and 5.0 and between 0 and 27.5 in the salmon farm (Fig.  
304 3).

305 No significant differences were found between the values of  $\alpha$  between outside and  
306 in the salmon farm for both macrofauna and foraminifera assemblages (t-test,  $p > 0.05$ ). In

307 turn, the parameter  $\phi$  differed significantly between outside and in the salmon farm (t-test,  
308  $p < 0.05$ ), for both macrofaunal and foraminiferal assemblages.

309



310

311 Figure 3: Values of the parameters  $\alpha$  and  $\phi$  characterizing the rank-frequency diagrams of  
312 macrofaunal (black dots) and foraminiferal (grey dots) assemblages in the Rade of Cherbourg  
313 outside the farm (A) and in the salmon farm (B).

314

315 Outside the farm, the parameters  $\alpha$  and  $\phi$  were highly significantly linearly correlated  
316 for foraminifera, *i.e.*  $\phi= 7.21$  and  $\alpha=10.6$ , while no significant correlation was found for  
317 macrofaunal assemblages. In the salmon farm, the parameters  $\alpha$  and  $\phi$  were significantly  
318 linearly correlated for foraminifera ( $\phi= 1.96$  and  $\alpha=2.48$ ) and macrofauna ( $\phi= 1.99$  and  $\alpha=$   
319  $3.07$ ). Finally, no significant differences were found between the slopes of the linear  
320 regression observed in the salmon farm for both foraminifera and macrofauna (ANCOVA,  $p >$   
321  $0.05$ ), but were both highly significantly smaller than the one observed outside the farm  
322 (modified t-test,  $p < 0.01$ ).

323

324

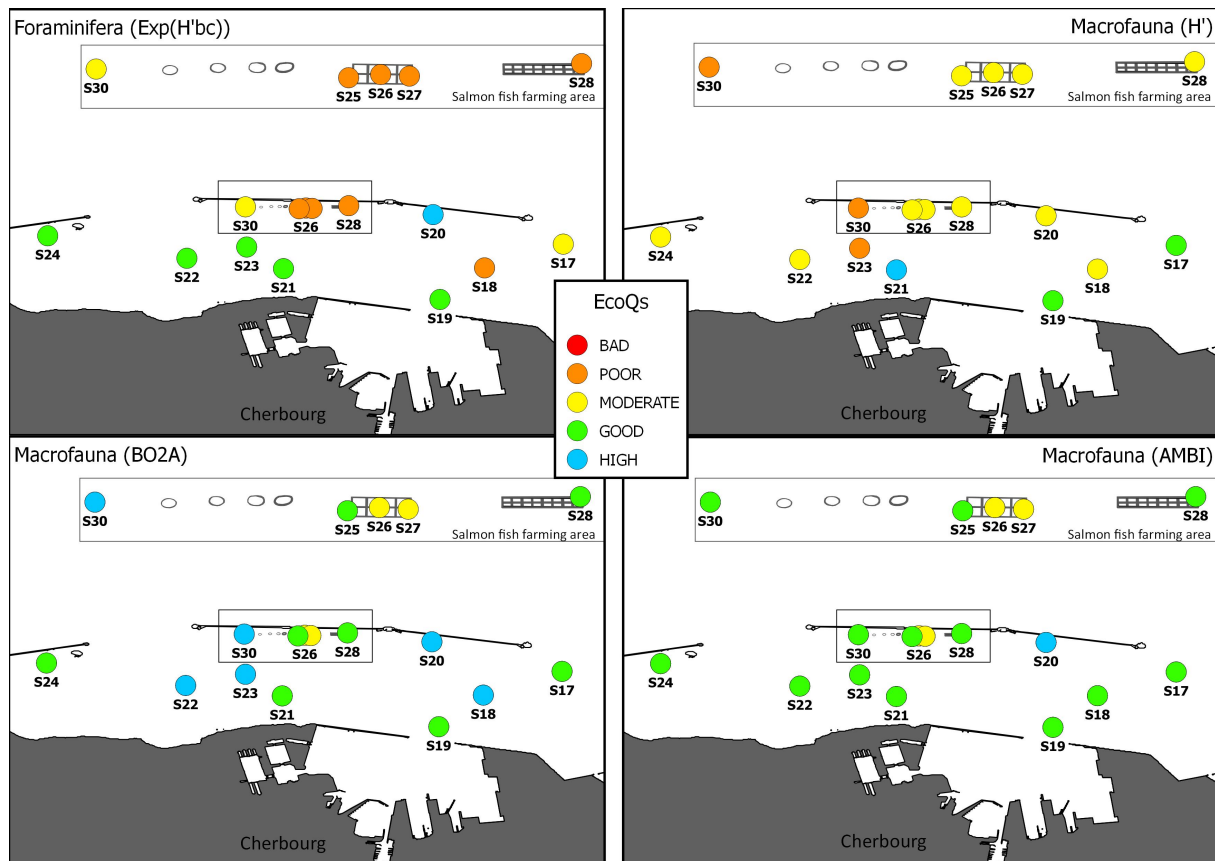
### 3.4 Ecological quality statuses

325

326 Diversity of benthic foraminifera measured by  $\text{Exp}(H'_{bc})$  was significantly lower in the salmon  
327 farm compare to the stations outside the farm (Table 2, t-test,  $p < 0.05$ ). Ecological quality  
328 statuses ranged between poor and high in the non-impacted stations, and between  
329 moderate to poor under the cages (Fig. 4). Outside the salmon farm, the two stations in  
330 moderate and poor EcoQs (S17 and S18) are located in the channel connecting the RdC with  
331 the open sea (Fig. 4).

332 There were no significant differences between the stations outside and inside the  
333 salmon farm for the diversity index  $H'$  (Table 2, t-test,  $p > 0.05$ ) and the sensitivity index  
334 BO2A (Table 2, t-test,  $p > 0.05$ ) calculated on the macrofauna data. Values of AMBI index  
335 were significantly higher inside the salmon (Table 2, t-test,  $p < 0.01$ ). Based on  $H'$ , EcoQs  
336 ranged between high and poor outside the farm, being mostly moderate; and between poor  
337 to moderate under the cages (Fig. 4). Indices BO2A and AMBI classified stations outside the  
338 farm as good to high, and between high to moderate inside the salmon farm (Fig. 4).

339



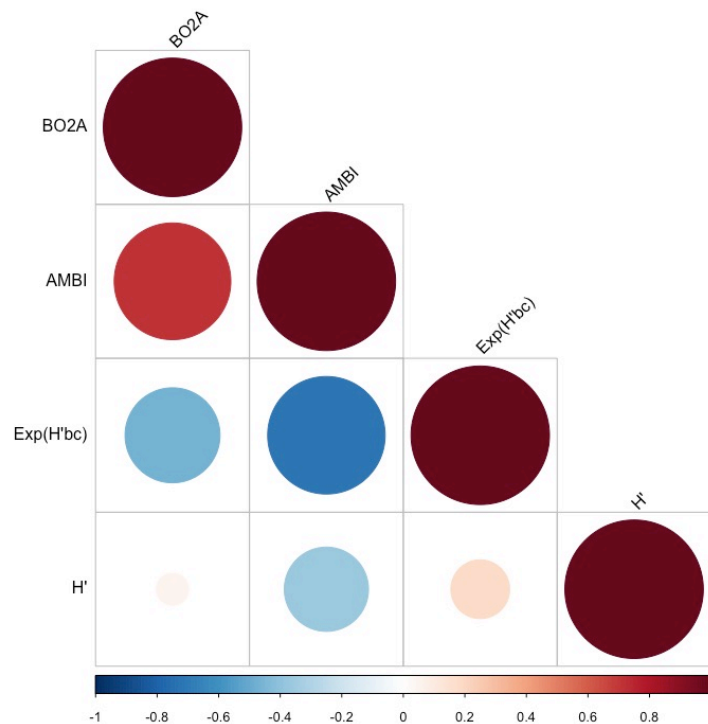
340

341 Figure 4: Ecological quality status in the RdC according to A- Foraminifera – Exp(H'bc), B-  
 342 Macrofauna – H', C- Macrofauna – BO2A and D- Macrofauna – AMBI.

343

344 The assessment of EcoQs partially mostly matched between benthic foraminifera and  
 345 macrofauna in the area outside the salmon farm to state that it was a nearly undisturbed  
 346 zone (Fig. 4). In the salmon farm, benthic foraminifera clearly showed that the whole area  
 347 was disturbed, while it was partly impacted based on macrofauna. Specifically, the Exp(H'bc)  
 348 based on benthic foraminifera was only significantly correlated with AMBI based on  
 349 macrofauna (Fig. 5,  $r=0.72$ ,  $p < 0.01$ ), AMBI was also significantly correlated to the BO2A  
 350 index (Fig. 5,  $r= 0.71$ ,  $p < 0.01$ ), all the other correlations were not significant (Fig. 5,  $p >$   
 351 0.05).

352



353

354 Figure 5: Correlation matrix for biotic indices (correlations are given as colour: blue are  
 355 positive and red negative).

356

#### 357 4. Discussion

358

##### 359 4.1 Effects of salmon fish farm on the benthic ecosystem health in the RdC

360

361 Our results did not reveal any specific accumulation of silt sediments nor organic  
 362 matter due to the presence of the salmon farm in the RdC. This fact confirmed previous  
 363 studies that showed only a moderate impact on the sediment characteristics in the RdC  
 364 (Dauvin et al., 2020; Kempf et al., 2002). Specifically, the semi-diurnal megatidal regime of  
 365 the Rade de Cherbourg (5 to 8 m tidal range from neap to spring tide) likely limits any long  
 366 term accumulation of OM under the cages, with tidal current velocities ranging between 10  
 367 and 70 cm s<sup>-1</sup> in the RdC in the salmon farm area (Dauvin et al., 2020). These velocities are

368 above the threshold of  $8 \text{ cm s}^{-1}$  that typically leads to the dispersal of excess organic waste  
369 (Yokoyama et al., 2006). Local anaerobic degradation by sulphate-reducing processes at the  
370 water-sediment interface, as well as high ammonium flux under the cages, resulting from  
371 faeces and waste-feed was nevertheless observed at the station under the cages in the RdC  
372 (Bachelet, 2014). Hence, although the impact in the RdC seems to be limited, the presence  
373 of the salmon fish farm affects the benthic habitat.

374         The areas outside the farm and under the cages in the Rade de Cherbourg were  
375 clearly discriminated by  $\text{Exp}(H'_{bc})$  based on benthic foraminifera. The EcoQs were mostly  
376 good in the non-impacted stations. Furthermore, the parameter  $\phi$  characterising the  
377 curvature of rank-frequency diagrams was much higher for the non-impacted stations. It  
378 suggested that the most abundant species had similar abundance (Seuront, 2013), hence  
379 confirming that benthic foraminiferal communities were in good health (Frontier, 1985,  
380 1976). At these stations, there were 15 species that reached at least 5% of relative  
381 abundances showing the diversity of the foraminiferal assemblages. Sensitive species like  
382 *Discorbis vilardeboanus* (Bouchet, 2007), *Criboelphidium magellanicum* (Armynot et al.  
383 2004; Francescangeli, 2017) and *Bolivina pseudoplicata* (Alves Martins et al., 2009) mostly  
384 occurred outside the farm, while their relative abundances dropped under the cages.

385         However, stations S17 and S18, though, situated outside the influence of the salmon  
386 fish farm, exhibited degraded ecological conditions with, respectively, moderate and poor  
387 EcoQS. These stations are in the channel that connects the RdC with the English Channel, the  
388 benthic habitat being mostly composed of fine sand with gravels and coarse sand (Baux et  
389 al., 2017). This is typical of high-energy hydrodynamic system. This type of habitat prevents  
390 for the establishment of a well diverse benthic foraminiferal communities, mainly because  
391 they prefer finer sediments (Murray, 2006). Hence, these stations are largely dominated by

392 *Quinqueloculina stelligera*, *Miliolinella subrotunda* and *Tricoculina oblunga*, porcellaneous  
393 foraminifera typically flourishing in sandy sediments (Murray, 2006).

394 Under the salmon cages, EcoQs were moderate to poor, showing a clear impact of  
395 the farm on benthic foraminiferal communities. At these stations, only the tolerant species  
396 *Bolivina variabilis* (Debenay et al. 2001; Armynot et al. 2009) and *Quinqueloculina stelligera*  
397 (Bergamin et al. 2003; Armynot du Châtelet, 2003; Jorissen et al. 2018) remained highly  
398 abundant in the foraminiferal assemblages. These observations were consistent with EcoQs  
399 obtained with benthic macrofauna in the same area (Dauvin et al., 2020). The presence of  
400 fish cages is largely acknowledged to induce deleterious effects on benthic communities  
401 (Angel et al., 2000; Karakassis et al., 2002; Dauvin et al., 2020), and this study did not make  
402 exception. Specifically, tolerant benthic foraminiferal species colonise sediments in fish  
403 farming areas (Angel et al., 2000; Damak et al., 2020; Pochon et al., 2015; Vidovic et al.,  
404 2009), and diversity would decrease. This is mostly due to the accumulation of organic  
405 matter that leads to anoxia in sediments under fish farm cages (Porrello et al., 2005;  
406 Yokoyama et al., 2006).

407 In conclusion, the strongest impact of the salmon farm in the RdC was restricted in  
408 the area directly under the cages, this impact being fairly moderated. In general, the effects  
409 of aquaculture are mostly limited in the sediment under the fish farms (Angel et al., 2000;  
410 Black, 2001; La Rosa et al., 2004). The abundances of tolerant and sensitive species were  
411 clearly constrained by the presence of salmon cages; tolerant species were more abundant  
412 in the assemblages impacted by the farm and, conversely, sensitive species were more  
413 abundant outside the fish farm.

414

415 **4.2 Benthic foraminifera as a reliable biological quality element?**



416

417           In contrast to numerous studies that showed a significant correlation between  
418 foraminiferal and macrofaunal diversity indices (Alve et al., 2019; Bouchet et al., 2018a;  
419 Mojtahid et al., 2008; Wlodarska-Kowalczyk et al., 2013),  $\text{Exp}(H'_{bc})$  based on benthic  
420 foraminifera was only significantly correlated with the AMBI index. When comparing EcoQs,  
421 we reported differences between results obtained with foraminifera diversity-based index  
422 and macrofaunal sensitivity-based indices. In the Firth of Clyde, while similar diversity ( $H'$ )  
423 patterns were reported, patent differences in sensitivity-based indices had also been  
424 observed (Mojtahid et al. 2008). In a study on the impact of oil-drill mud disposal, benthic  
425 foraminifera were more sensitive than macrofauna to environmental degradations  
426 (Denoyelle et al. 2010). Despite some discrepancies observed in our study, foraminifera and  
427 macrofauna showed similar trends *i.e.* a spatially limited and moderated degradation of  
428 EcoQs under the salmon cages. In the RdC, benthic foraminifera-based EcoQs classification  
429 were more severe than macrofauna to the presence of salmon farming, suggesting that may  
430 be more efficient to detect the deleterious effects of this anthropogenic pressure. It further  
431 confirms that benthic foraminifera are reliable indicators of fish farming impacts (Damak et  
432 al., 2020; Pochon et al., 2015; Vidovic et al., 2009).

433           Foraminifera have increasingly been used in environmental monitoring studies (e.g.  
434 Alve et al., 2019; Bouchet et al., 2018a; Damak et al., 2020; Pochon et al., 2015). However,  
435 they are still not officially acknowledged as a biological quality element within the European  
436 water framework directive. This study further stressed the fact that foraminifera can be  
437 considered as a reliable biological quality element to assess EcoQs in marine systems.  
438 Specifically, the use of the diversity index  $\text{Exp}(H'_{bc})$  based on benthic foraminifera showed  
439 good performance in detecting the impact of salmon farming in RdC. This method was

440 originally tested along a decreasing gradient of bottom-water oxygen concentration in SE  
441 Norwegian fjords (Bouchet et al., 2012). In the present work, we showed its relevance to  
442 assess the impact of salmon fish farm. It further confirms previous findings (Bouchet et al.,  
443 2018a; Dijkstra et al., 2017; Francescangeli et al., 2016; Melis et al., 2016) showing that  
444  $\text{Exp}(H'_{bc})$  based on benthic foraminifera is accurate to assess the impact of different sources  
445 of stress and in different types of marine ecosystems. Decreasing abundances of sensitive  
446 species associated to increasing abundances of tolerant species under the cages in the RdC  
447 further showed the potential of benthic to serve as ecological sentinels of environmental  
448 degradations.

449         Sensitivity-based indices are actually largely prioritized over diversity ones for benthic  
450 macrofauna (Borja et al., 2000; Bouchet and Sauriau, 2008; Dauvin, 2018; Dauvin and  
451 Ruellet, 2009; Muxika et al., 2005; Pinto et al., 2009, 2009). Lately, the development of  
452 sensitivity based indices for benthic foraminifera like the Foram-AMBI (Alve et al. 2016;  
453 Jorissen et al. 2018), the FSI index (Dimiza et al., 2016) or the TSI-med (Barras et al., 2014),  
454 led to the prioritization of this type indices instead of diversity ones (Musco et al., 2017;  
455 Gomez-Leon et al., 2018; Damak et al., 2020; El Kateb et al., 2020). However, the “indicator  
456 species” concept, which is the theoretical background of sensitivity indices, had recently  
457 been questioned for macrofauna (Dauvin et al., 2010; Spilmont, 2013; Zettler et al., 2013),  
458 since the tolerance spectrum of species might be wider and more complex than a simple  
459 categorisation, *i.e.* “sensitive species” or “tolerant species”. It means that sensitivity-based  
460 indices like AMBI might be cautiously used. In this study, diversity (*i.e.*  $\text{Exp}(H'_{bc})$ ) proved to  
461 be accurate to assess EcoQs in the RdC based on benthic foraminifera. Hence, diversity itself  
462 must not yet be excluded from monitoring survey.

463         This study further aimed at testing RFDs, a method describing diversity, to assess the

464 health of benthic communities. Despite their early development and application to  
465 planktonic organisms such as tintinids (Margalef, 1957), chaetognaths and petropods  
466 (Frontier and Bour, 1976; Frontier, 1977), rank-frequency diagrams appeared to be well  
467 adapted to detect changes in benthic communities (Warwick and Clark, 1995); both on  
468 macrofauna (Sanvicente-Anorve et al., 2002) and foraminifera (Bouchet et al., 2007). In this  
469 work, RFDs appeared well adapted to identify differences in benthic foraminiferal and  
470 macrofaunal communities between the area impacted by the salmon farm and the non-  
471 impacted stations. Following the theoretical work of Frontier (1985, 1976), benthic  
472 communities were at stage 2 corresponding to a mature stage outside the salmon farm,  
473 hence equivalent to a good or high EcoQ, while it was at stage 1 under the salmon farm  
474 highlighting that communities were under pressure, hence an EcoQ between moderate and  
475 bad. This study suggests that RFDs should be included in the general reflexion on the  
476 evaluation of the good environmental status (GES) within the European marine strategic  
477 framework directive (MSFD). Conversely to the water framework directive, which enforced  
478 to follow a chart of five EcoQs, the MSFD aims at achieving GES, *i.e.* to differentiate between  
479 undisturbed and disturbed areas.

480

## 481 **5. Conclusion**

482

483         This work confirmed that benthic foraminifera are reliable to evaluate EcoQs in the  
484 context of salmon fish farm as the indices used for the macrofauna. Hence, this study  
485 represents a further step towards the official inclusion of benthic foraminifera in the list of  
486 biological quality elements. Comparison with macrofauna confirmed that these two groups,  
487 that share similar features, have the same response to pollution. However, differences in the

488 reported EcoQs might suggest that inter-calibration between foraminiferal and macrofaunal  
489 indices should be developed in the future. The present work warrants the need to promote  
490 studies and surveys including at the same time foraminifera and macrofauna samplings.  
491 Furthermore, the assessment of diversity by mean of  $\text{ExpH}'_{bc}$  and RFD is relevant to assess  
492 the health of benthic communities, although it had been subject to controversy. More credit  
493 should be given to these methods in environmental monitoring studies in the future.

494

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502

503

504

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