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

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Abstract

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

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

1. Introduction

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

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

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

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

Benthic foraminifera have increasingly been acknowledged as indicators of human-induced stresses (e.g. Alve, 1995; Francescangeli et al., 2016; Polovodova Asteman et al., 2015), such as oil spills (Morvan et al., 2004), heavy metals (Armynot du Châtelet et al., 2004), urban sewage (Melis et al., 2016), and aquaculture (Bouchet et al., 2007; Debenay et al., 2015; Vidovic et al., 2014). Specifically, fish farms induce clear shifts in the community structure of benthic foraminifera (Pochon et al., 2015), promote tolerant species (Angel et al., 2000) and lead to moderate to poor EcoQs (Bouchet et al., 2018a). In Norwegian fjords, benthic foraminiferal communities significantly correlate with benthic macrofauna communities, indicating that foraminifera can also be considered as good indicators of environmental conditions (Bouchet et al., 2018b). Foraminiferal indices based either on diversity (Alve et al., 2009; Bouchet et al., 2013, 2012) and on the sensitivity of species to organic pollution (Alve et al., 2016; Barras et al., 2014; Dimiza et al., 2016; Jorissen et al., 2018), have been designed and successfully applied to assess EcoQs of benthic habitats (Alve et al., 2019; Bouchet et al., 2018a, 2012; Damak et al., 2020; Dijkstra et al., 2017; Dolven et al., 2013; El Kateb et al., 2020; Francescangeli et al., 2016; Melis et al., 2016). Alve et al. (2019) further demonstrated that foraminifera and macrofauna have similar indicator efficiency by applying multimetric index based on foraminifera (NQIf) as an alternative, which is an adaptation of the Norwegian Quality Index (NQI), an internationally intercalibrated macrofauna index. Based on their results, Alve et al. (2019) recommended the inclusion of foraminifera as Biological Quality Element within the European Water Framework Directive’s guidelines (WFD). In a study of Italian transitional waters, benthic foraminifera have further been shown to be more accurate than benthic macrofauna to assess EcoQs (Bouchet et al., 2018a). Considering the difficulties to assess EcoQs in naturally stressed ecosystems (Dauvin, 2007; Dauvin and Ruellet, 2009; Elliott and Quintino, 2007), foraminifera may be a relevant alternative to macrofauna (Hess et al., in press). Because of their potential to reconstruct palaeo-environments (Alve, 1991; Alve et al., 2009; Hayward et al., 2004), an unique feature compared to benthic macrofauna (except for molluscs; Poirier et al., 2009), foraminifera are also good candidates to establish objective and reliable reference conditions (Dolven et al., 2013; Francescangeli et al., 2016). In short, benthic foraminifera may be a relevant complement to benthic macrofauna to assess EcoQs. These two groups exhibit similar features. Both are benthic and sedentary organisms, their distribution patterns depending directly on the environmental conditions. Furthermore, foraminiferal and macrofaunal community compositions in SE Norwegian fjords were significantly correlated suggesting that benthic foraminiferal distribution patterns mirror those of benthic macrofauna (Bouchet et al., 2018b). Intercalibration of benthic foraminiferal and macrofaunal biotic indices further confirm the complementarity of these two groups (Alve et al., 2019). Hence, this study was designed to discuss the relative strength of the classical macrofauna tool and the “newcomers” benthic foraminifera one to assess ecological quality statuses in the RdC in the context of salmon fish farming.

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

2. Materials and Methods

2.1 Sedimentological, foraminiferal and macrofaunal sampling

For the present study, thirteen stations (Fig. 1) were sampled in June 2015 in the RdC by scuba-diving using a push corer (diameter: 9.8 cm, layer: 0-1 cm, n = 3). Stations from S17 to S24 were away the salmon fish farm in the RdC, while stations from S25 to S28 and S30 were directly under the salmon cages (Fig. 1). The geographical positions of the stations were determined using a RTK Global Position System (GPS ASHTECH Promark 120, accuracy better than 10cm). The study stations were visited for benthic macrofauna sampling in February 2014 at stations S17 to S22 and in February 2015 at stations S23 to S28 and S30. The sampling strategy used for the study of the macrobenthic communities is described in detail in Baux et al. (2017). Benthic macrofauna raw data are published in Baux et al. (2017) and Andres et al. (2020).

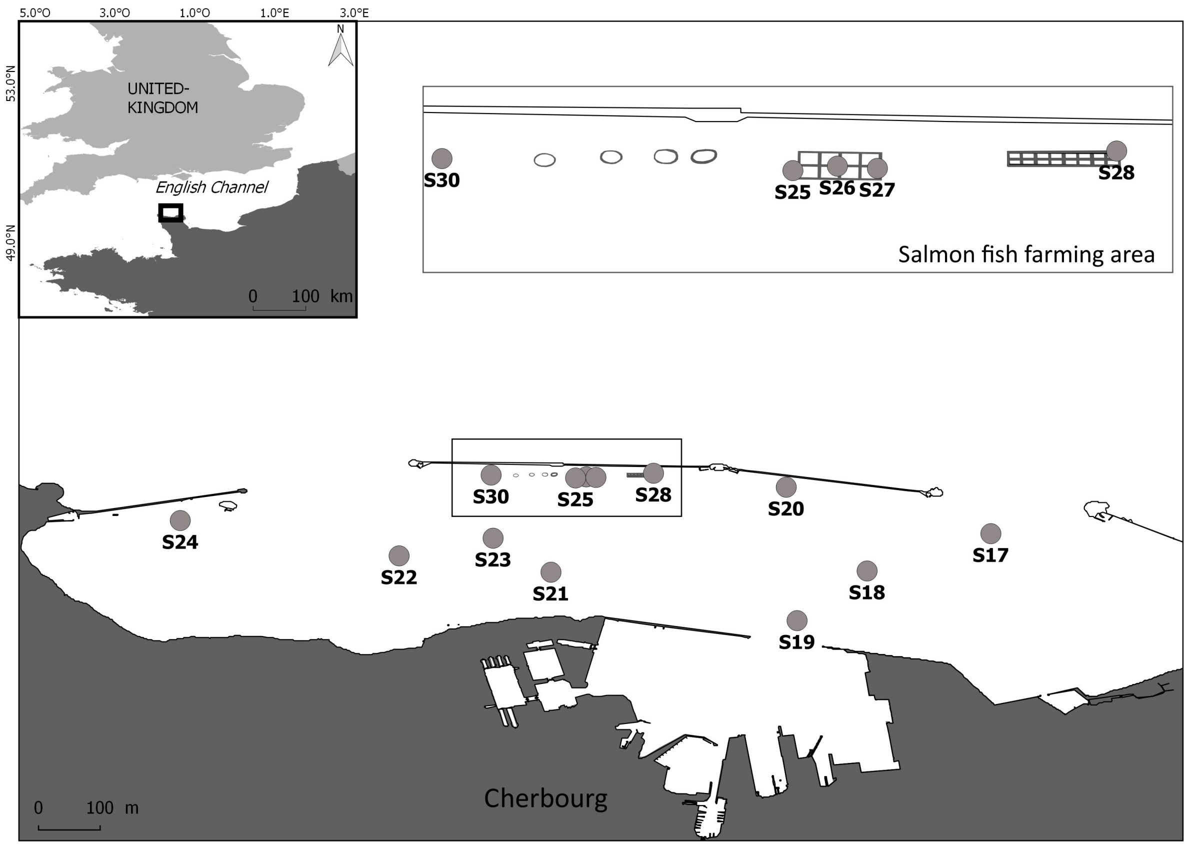

Figure 1: Sampling sites in the Rade de Cherbourg for benthic foraminifera (this study) and benthic macrofauna (Baux et al. 2017), sites are labelled following Baux et al. (2017).

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

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

2.2 Fine fraction analysis

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

2.3 Organic matter analysis

Before geochemical analysis, the fine fraction <63 µm was crushed and homogenized for each sample. Total organic carbon (TOC) contents were measured by combustion in a LECO CS 300. Three replicates of dried and homogenized sediment (50 mg) were analysed per station. Samples were heated to 1,600 °C and the amount of CO_2 was measured by infrared absorption. For the analysis of Total Organic Carbon (TOC), sediment samples were acidified by HCl (12.5%) to remove carbonates. All inorganic carbon was assumed to be in the form of calcium carbonate. Quality control was maintained by measuring LECO certified reference materials (see further details on the method used for organic matter analysis in Baux et al., 2019).

2.4 Foraminiferal analysis

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

2.5 Data analyses

2.5.1 Rank-frequency diagram

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

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

where f_s is the frequency (i.e. relative abundance) of a species of rank r (the most abundant species has a rank r= 1, the second more abundant a rank r= 2, and so on, and the less abundant species has a rank $r = N$, where N is the number of species found in a sample), f is a pre-factor based on the overall abundance of a community, and α and ϕ fitting parameter describing the diversity and evenness of a given community. Rank-frequency diagrams consist of frequencies of species plotted against their respective ranks organized in decreasing order, and with both axes in logarithmic scale (see examples in Fig. 2).

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

2.5.2 Ecological Quality Statuses calculation

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

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

EcoQS and associated colour code	Bad	Poor	Moderate	Good	High
Foraminifera - 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

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

2.5.3 Statistical analysis

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

3. Results

3.1 Environmental parameters

Stations		Sediment		Biotic Indices			
		<63 (%)	TOC (%)	Foraminifera	Macrofauna		
				$Exp(H'_{bc})$	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

Table 2: Grain size (fraction <63 μm), total organic carbon content (%) and biotic indices values at sampling stations.

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

3.2 Foraminiferal main species

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

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

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

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

		Outside the farm								Salmon farm				
		S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S30
Agglutinated	*Remaneica helgolandica*	0.0	0.0	0.0	0.0	0.0	**7.5**	3.7	0.2	1.0	0.0	0.0	0.0	0.8
	Textularia truncata	0.0	0.0	0.0	0.0	0.5	**6.8**	**6.9**	0.0	0.0	0.0	0.0	0.0	0.0
Porcellaneous	*Adelosina bicornis*	2.0	2.6	1.4	0.4	**6.9**	1.9	3.7	0.7	0.0	3.4	0.8	1.4	2.4
	Adelosina dubia	3.9	1.3	1.8	3.5	4.8	0.4	2.1	1.8	0.0	**5.1**	0.8	4.1	2.4
	Miliolinella subrotunda	**18.6**	1.3	0.0	**5.1**	0.0	3.0	0.5	0.9	0.0	**8.5**	0.0	**5.9**	0.0
	Quinquelocina stelligera	**30.4**	**50.7**	**12.6**	**7.1**	**22.9**	**7.1**	**17.6**	**20.5**	**38.0**	**44.9**	**43.7**	**40.0**	**22.6**
	Quinqueloculina sp.	0.0	2.6	0.0	0.0	0.5	0.0	0.0	0.0	**27.8**	0.8	0.0	0.9	0.0
	Triloculina oblonga	**9.8**	2.0	2.3	**5.5**	3.2	4.5	0.0	3.0	1.5	0.0	0.8	2.3	**6.5**
	Triloculina triloculina	4.9	**7.9**	0.5	0.0	4.8	1.1	1.1	0.3	0.0	2.5	2.4	**6.4**	0.8
Hyalin	*Bolivina pseudoplicata*	1.0	4.6	**16.7**	**9.8**	**14.4**	**12.8**	4.8	**10.2**	**5.4**	3.4	**7.9**	2.3	**8.1**
	Bolivina variabilis	2.9	**10.5**	**21.6**	**16.9**	**14.9**	**7.9**	**11.2**	**20.5**	**9.3**	**11.0**	**23.0**	**13.2**	**25.8**
	Bulimina elegans	2.0	1.3	**6.3**	3.5	0.5	1.5	0.0	1.5	0.0	1.7	0.8	0.9	1.6
	Criborelphidium gunteri	0.0	0.0	0.9	2.4	0.0	0.0	0.0	**6.2**	0.0	0.0	0.0	0.0	0.0
	Cribroelphidium magellanicum	3.9	4.6	4.1	**7.1**	1.6	4.9	**8.0**	2.8	3.4	1.7	0.0	**6.8**	4.8
	Discorbis vilardeboanus	0.0	0.0	1.8	2.4	4.3	**14.7**	**20.2**	0.7	1.5	0.8	2.4	0.0	4.0
	Hopkinsina atlantica	0.0	0.7	3.6	**6.3**	2.1	0.0	0.0	**10.3**	0.0	0.8	0.8	0.5	4.8
	Nonionella sp.1	**5.9**	0.7	**6.8**	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

Table 3: Relative abundances of the main foraminiferal species (>5%).

3.3 Rank-frequency diagrams

The parameters α and ϕ allowed for a synthetically analysis of the rank-frequency distributions (Fig. 2).

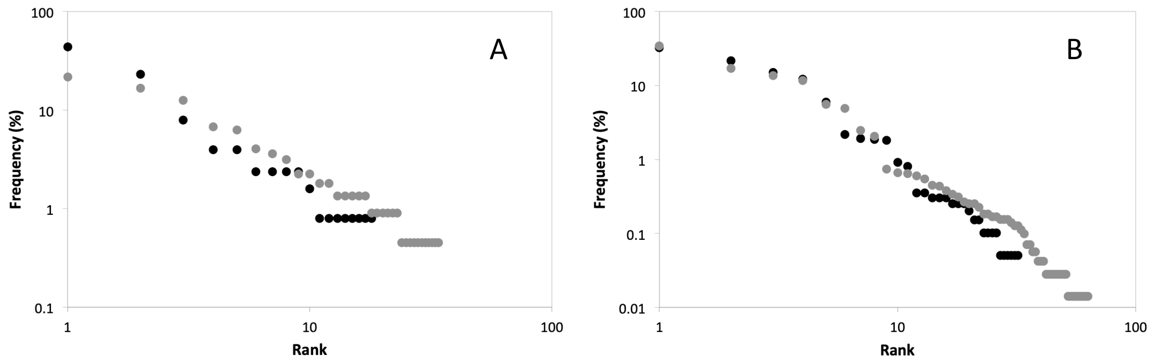

Figure 2: Typical examples of rank-frequency distributions of benthic foraminifera (A) and macrofauna (B) in the Rade de Cherbourg outside the farm (grey dots, station S19) and in the salmon farm (black dots, station S27).

For foraminiferal assemblages, the parameters α and ϕ respectively ranged between 1.82 and 3.57 and between 0 and 4.2 outside the farm, and between -0.3 and 2.3 and between 1.19 and 2.04 in the salmon farm (Fig. 3). For macrofaunal assemblages, the parameters α and ϕ respectively, ranged between 2.5 and 3.7 and between 2.2 and 17.5 outside the farm, and between 1.25 and 5.0 and between 0 and 27.5 in the salmon farm (Fig. 3).

No significant differences were found between the values of α between outside and in the salmon farm for both macrofauna and foraminifera assemblages (t-test, $p > 0.05$). In turn, the parameter ϕ differed significantly between outside and in the salmon farm (t-test, $p < 0.05$), for both macrofaunal and foraminiferal assemblages.

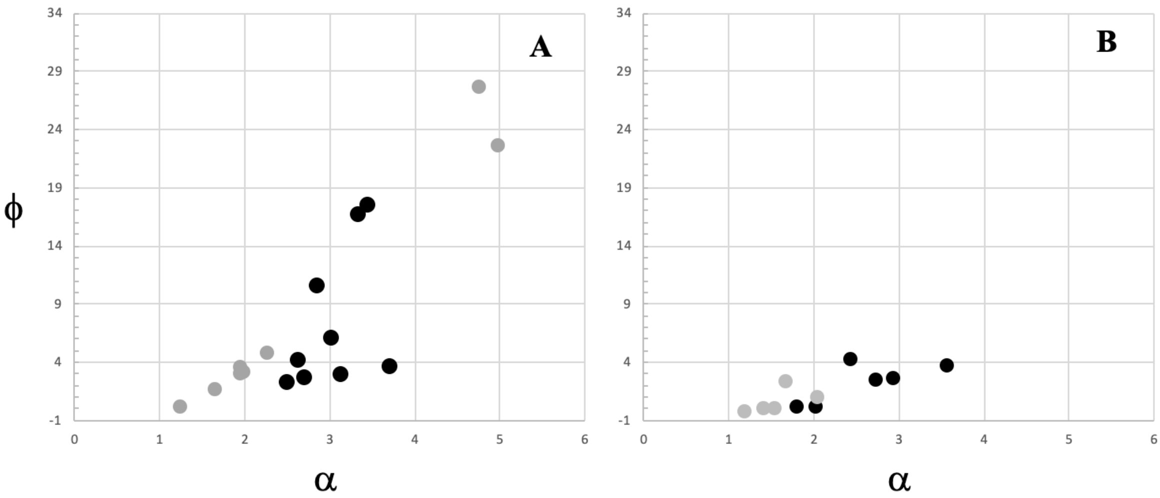

Figure 3: Values of the parameters α and ϕ characterizing the rank-frequency diagrams of macrofaunal (black dots) and foraminiferal (grey dots) assemblages in the Rade of Cherbourg outside the farm (A) and in the salmon farm (B).

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

3.4 Ecological quality statuses

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

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

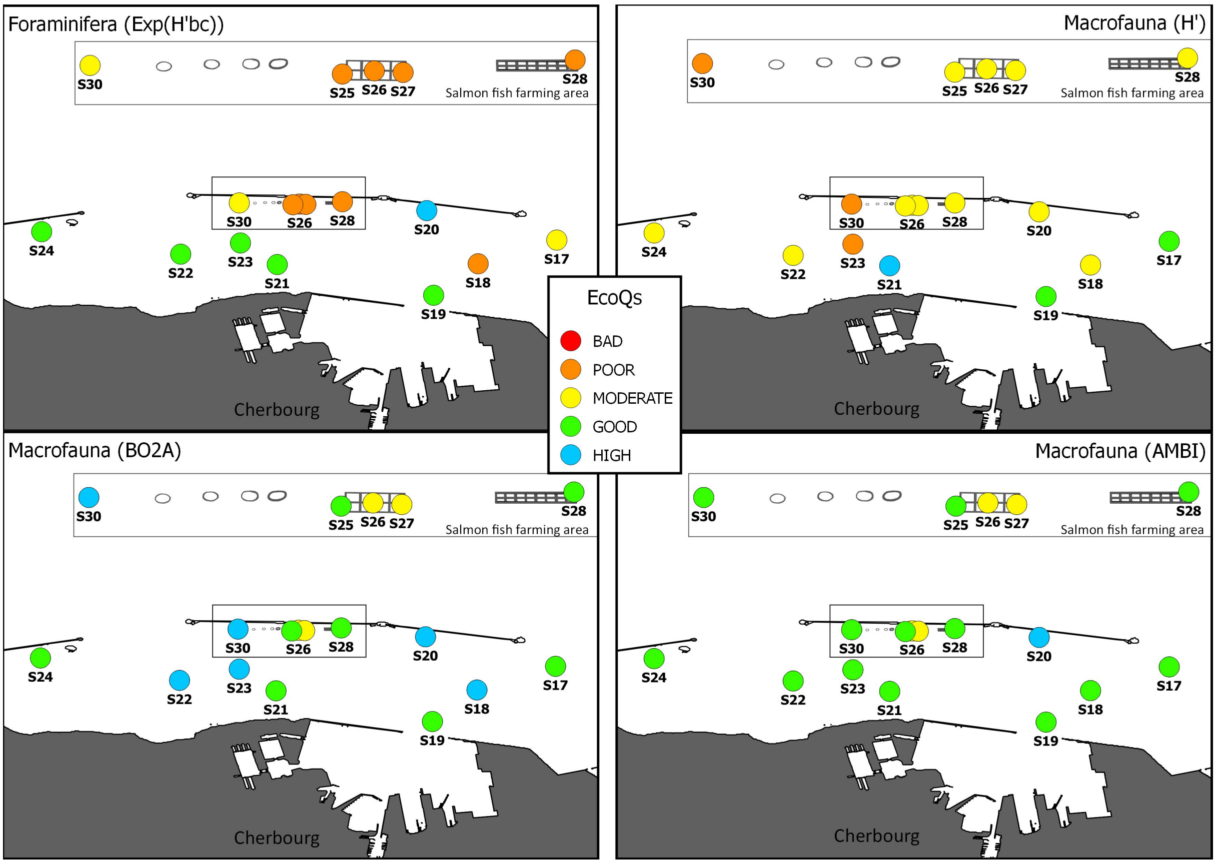

Figure 4: Ecological quality status in the RdC according to A- Foraminifera – Exp(H'_{bc}), B- Macrofauna – H', C- Macrofauna – BO2A and D- Macrofauna – AMBI.

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

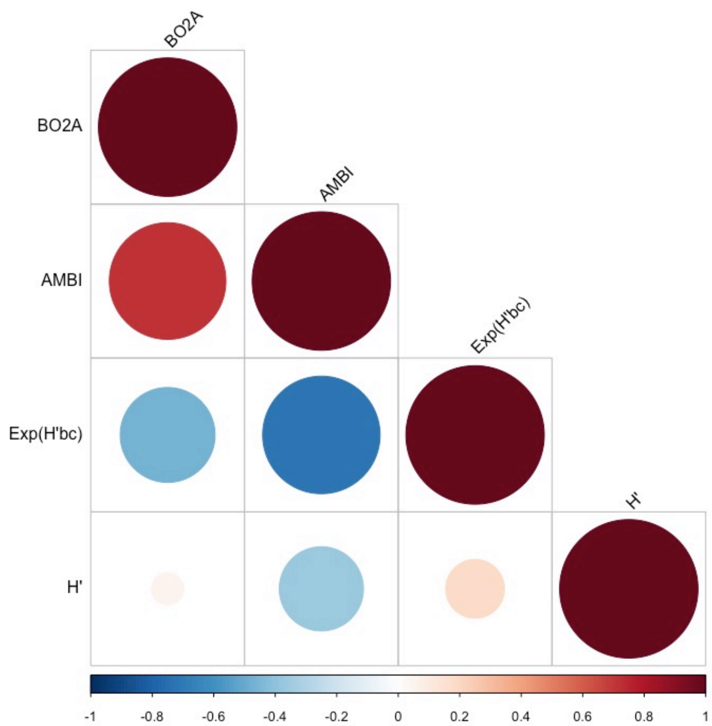

Figure 5: Correlation matrix for biotic indices (correlations are given as colour: blue are positive and red negative).

4. Discussion

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

Our results did not reveal any specific accumulation of silt sediments nor organic matter due to the presence of the salmon farm in the RdC. This fact confirmed previous studies that showed only a moderate impact on the sediment characteristics in the RdC (Dauvin et al., 2020; Kempf et al., 2002). Specifically, the semi-diurnal megatidal regime of the Rade de Cherbourg (5 to 8 m tidal range from neap to spring tide) likely limits any long term accumulation of OM under the cages, with tidal current velocities ranging between 10 and 70 cm s^{-1} in the RdC in the salmon farm area (Dauvin et al., 2020). These velocities are above the threshold of 8 cm s^{-1} that typically leads to the dispersal of excess organic waste (Yokoyama et al., 2006). Local anaerobic degradation by sulphate-reducing processes at the water-sediment interface, as well as high ammonium flux under the cages, resulting from faeces and waste-feed was nevertheless observed at the station under the cages in the RdC (Bachelet, 2014). Hence, although the impact in the RdC seems to be limited, the presence of the salmon fish farm affects the benthic habitat.

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

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

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

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

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

4.2 Benthic foraminifera as a reliable biological quality element?

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

Foraminifera have increasingly been used in environmental monitoring studies (e.g. Alve et al., 2019; Bouchet et al., 2018a; Damak et al., 2020; Pochon et al., 2015). However, they are still not officially acknowledged as a biological quality element within the European water framework directive. This study further stressed the fact that foraminifera can be considered as a reliable biological quality element to assess EcoQs in marine systems. Specifically, the use of the diversity index Exp(H'_{bc}) based on benthic foraminifera showed good performance in detecting the impact of salmon farming in RdC. This method was originally tested along a decreasing gradient of bottom-water oxygen concentration in SE Norwegian fjords (Bouchet et al., 2012). In the present work, we showed its relevance to assess the impact of salmon fish farm. It further confirms previous findings (Bouchet et al., 2018a; Dijkstra et al., 2017; Francescangeli et al., 2016; Melis et al., 2016) showing that Exp(H'_{bc}) based on benthic foraminifera is accurate to assess the impact of different sources of stress and in different types of marine ecosystems. Decreasing abundances of sensitive species associated to increasing abundances of tolerant species under the cages in the RdC further showed the potential of benthic to serve as ecological sentinels of environmental degradations.

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

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

5. Conclusion

This work confirmed that benthic foraminifera are reliable to evaluate EcoQs in the context of salmon fish farm as the indices used for the macrofauna. Hence, this study represents a further step towards the official inclusion of benthic foraminifera in the list of biological quality elements. Comparison with macrofauna confirmed that these two groups, that share similar features, have the same response to pollution. However, differences in the reported EcoQs might suggest that inter-calibration between foraminiferal and macrofaunal indices should be developed in the future. The present work warrants the need to promote studies and surveys including at the same time foraminifera and macrofauna samplings. Furthermore, the assessment of diversity by mean of $ExpH'_{bc}$ and RFD is relevant to assess the health of benthic communities, although it had been subject to controversy. More credit should be given to these methods in environmental monitoring studies in the future.

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