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## **Biomonitoring of *Mimachlamys varia* transplanted to areas impacted by human activities (La Rochelle Marina, France)**

Marine Breitwieser, Marine Barbarin, Christine Plumejeaud-Perreau, Emmanuel Dubillot, Thierry Guyot, Valérie Huet, Carine Churlaud, Thibaut Coulombier, Isabelle Brenon, Denis Fichet, et al.

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1 **Research paper**

2 **Title** Biomonitoring of *Mimachlamys varia* transplanted to areas impacted by human  
3 activities (La Rochelle Marina, France).

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42           **Abstract**

43           The development of human activities on French Atlantic coastlines (La Rochelle) lead to  
44 chronic pollution of the environment by organic (pesticides, hydrocarbons, agrochemicals) and  
45 inorganic (heavy metals) contaminants. These past years, several regulations have been implemented  
46 to preserve coastal environments. The purpose of this study was to perform biomonitoring of bivalve  
47 species using an outdoor caging technique. The goal of our work was to assess the impact of  
48 harbour's trace elements on the state of health of the marine bivalve *Mimachlamys varia*. First, various  
49 molecular defence biomarkers were measured: SOD (oxidative stress), GST (detoxification process),  
50 MDA (lipid peroxidation), and Laccase (immune reaction). Thus, in April 2016, scallops were  
51 collected at three caging sites, which differ by their levels of pollution, after transplantation into port  
52 areas (fairing, rainwater) and a control site (marsh). Bivalve samples were taken at three sampling  
53 dates (D0, D07, D21). Biomarker assays were performed in the digestive glands due to their  
54 bioaccumulation properties. The second aim was to explore the impacts of inorganic pollutants placed  
55 in environmental harbour's sites. After 21 days, the biomarker response of transplanted bivalves  
56 revealed a SOD decrease, Laccase and GST stimulations, higher concentrations in Cu, Fe, As, Co,  
57 Mn, Zn, Sn and no significant variation of MDA concentration. Our ecological relevance of biomarker  
58 approaches opens interesting perspectives to identify *M. varia* such as a pertinent marine sentinel  
59 species. The several selected biomarkers determined could confirm their ability to appraise the water  
60 quality of hydro-systems located in French coastlines, such as port areas.

61   **Keywords:** biomarkers, environmental quality, heavy metals, port area

62

63 **1. Introduction**

64 Over the years, concerns have been raised about heavy metal contaminations, particularly in port  
65 areas and their surroundings. The human activities and downtown locations, which have direct access  
66 to the sea, could generate different types of wastes released in the environment as dissolved particles,  
67 macro-wastes and liquid effluent. These sea wastewaters reveal the presence of a mixture of chemical  
68 contaminants in significant amount, including heavy metals, polycyclic aromatic hydrocarbons  
69 (PAHs), pesticides, polychlorobiphenyl (PCBs) and other chemicals (Dorris et al., 1972). Most of  
70 them are toxic, even at low concentrations (Long et al., 1995). Therefore, environmental  
71 contamination is a major threat to marine ecosystems and human health (Fasulo et al., 2012a; Sureda  
72 et al., 2011), particularly near harbours and cities.

73 One of the largest harbour sites in Europe is the marina of La Rochelle. The harbour stretches  
74 from the marina to 15 km of docks along the coastline of La Rochelle (France). This harbour, settled  
75 in the 1970s, has today 5,500 spaces and welcomes 160 companies of the nautical sector, as a result of  
76 urban and economic developments.

77 Previous reports published by CREOCEAN (2016) and recent studies performed in Bay of  
78 Biscay (Milinkovitch et al., 2015; Breitwieser et al., 2017) revealed that Cu, Cd, As and trace elements  
79 are found in high concentrations, exceeding the standard limit reported by national and international  
80 sediment quality guidelines (Low n° 1881/2006). Moreover, different projects documented the key  
81 role played by cities and harbours in the contamination of the coastline. The Charente-Maritime area  
82 has acknowledged the importance of environmental state of health and was recognised as an  
83 outstanding functional set on the Atlantic coast by the French Environmental Protection Ministry in  
84 2006. Indeed, this marine region is a mudflat ecosystem full of various commercialised organisms  
85 such as bivalve species. Bivalve molluscs – in particular mussels, oysters and variegated scallops – are  
86 widely used as bio-indicators in environmental monitoring programmes (Mussel Watch Program,  
87 IFREMER networks) due to their large geographical distribution. In addition, these filter-feeding  
88 organisms have a sedentary lifestyle, which enables them to bioaccumulate and tolerate great amounts

89 of pollutants in their tissues (Lacroix et al., 2015; Sureda et al., 2011; Luna-Acosta et al. 2010;  
90 Bustamante et al., 2005). In addition, as mussels, black scallops *Mimachlamys varia* are suitable to  
91 conduct caging experiments in port areas (active biomonitoring). *Mimachlamys varia* is located in  
92 water/sediment interface, which is a highly relevant area to assess sediment quality. Our approach  
93 allows a reduction of physiological variables by using variegated scallops from a single hatchery  
94 population, more controlled over the experiment, and an accurate evaluation of the effects of  
95 polymetallic contamination in natural environments (Cappello et al., 2013a, 2013b, 2015; Fasulo et al.,  
96 2012b; Lacroix et al., 2015; Marigomez et al., 2013; Jimeno-Romero et al., 2019).

97         This work presents some of the results from the DYPOMAR research project. A multi-  
98 biomarker approach is performed on the digestive glands of scallops to assess the potential adverse  
99 effects of polymetallic contamination on black scallops caged in the La Rochelle marina. The bivalve  
100 digestive glands are involved in digestion, bioaccumulation and are one of the first organs to be  
101 affected by environmental stressors. Therefore, they are frequently used as a model system in  
102 ecotoxicological studies (Bustamante et al., 2005; Milinkovitch et al., 2015; Frantzen et al., 2016;  
103 Breitwieser et al., 2016; Luna-Acosta et al., 2017). Further studies describe that after pollutants  
104 exposure, bivalve's digestive tissues are involved in immune defence, detoxification processes and in  
105 homeostatic regulation (Marigomez et al., 2002; Moore and Allen, 2002), and histopathological  
106 alterations (Garmendia et al., 2011); so, digestive gland could correspond to a standardised tool for  
107 monitoring the general state of health of organisms (Amiard-Triquet et al., 2013) is assessed.

108         Several biomarkers (defence and damage) were used during the study to highlight the different  
109 reactions that can be induced after contamination. The defence biomarkers used are superoxide  
110 dismutase (SOD), a marker of oxidative stress, glutathione S-transferase (GST), involved in the  
111 process of detoxification of xenobiotics, and Laccase with a role in the modulation of immune  
112 response. The biomarker of damage, the malondialdehyde (MDA), is involved in lipid peroxidation  
113 process. Indeed, aquatic invertebrates may suffer from impairment in the enzymatic systems after  
114 being exposed to toxic compounds (Amiard-Triquet et al., 2013). The concentrations of heavy metals  
115 in the digestive glands are also measured to carry out a qualitative correlative approach. The quality

116 brings together several biometric parameters (length, thickness and width), using the following  
117 equation:

$$118 \quad \text{Quality} = \frac{\text{length} + \text{thickness}}{\text{width}}$$

119 This measure of quality is found in particular within the framework of the Red Label program (quality  
120 index) or biomonitoring measures to evaluate the health status of bivalves.

121 In addition, metallothionein concentrations were measured in other organisms at similar sampling  
122 dates to collect additional information on metal contamination at port sites (fairing, rainwater).  
123 Metallothionein is a defence biomarker that can be overexpressed in tissues in the presence of high  
124 metal concentrations (Damiens et al., 2006).

## 125 **2. Material and methods**

### 126 ***2.1 Sampling sites and caging transplantation procedure***

127 Two sampling sites located in the marina of La Rochelle (Nouvelle-Aquitaine region) and  
128 exposed to diffuse trace elements contamination are chosen. Sampling sites are named as follow: the  
129 careening area, exposed to contamination due to boat handling (antifouling paint residues, sacrificial  
130 anodes), and the rainwaters site, located near one of the biggest rainwaters outlets, which is  
131 characterised by a lack of filtration system (Figure 1). Previous studies revealed that the marina of La  
132 Rochelle contained high levels of heavy metals due to the port area being semi-closed (Breitwieser et  
133 al., 2016; Breitwieser et al., 2017). The third study site is a closed marsh of the laboratory, considered  
134 as the reference site, located a few kilometres away from the marina. In spring 2016, a batch of  
135 variegated intertidal scallop (*Mimachlamys varia*) from the Tinduff hatchery (Brittany Region) was  
136 caged in the marsh area for fifteen days in order to acclimate. Scallops were maintained one week in  
137 aerated seawater and then transplanted in selected sites in the two intra-harbour sites and the marsh. At  
138 each experiment day (D0, D07, D21), surviving species in the caging are counted and compared to the  
139 initial batch number (Fasulo et al., 2012b, Cappello et al. 2013a). A high mortality rate is observed  
140 during the acclimation period, likely due to the stress of transportation from Brittany to La Rochelle

141 (Maguire et al., 1999, Sarkis et al. 2005). In contrast, a low mortality rate (23%) is recorded after  
142 transplantation in the harbour sites. Ten scallops per site at two experiment dates (D07 and D21) are  
143 sampled and prepared on the spot for biochemical analysis and heavy metals quantification. Ten  
144 scallops are sampled as representative of D0 at the marsh site (Table 1). The dissections and the  
145 conservation in the fridge on site prevent from biological degradation.

## 146 ***2.2 Water physicochemical assessments***

147 From 17 June 2016 to 14 July 2017, temperature, salinity, turbidity, chlorophyll and dissolved  
148 oxygen are measured at 5min intervals using a multi-parameter probe (YSI 6600V2). Three  
149 monitoring stations, composed of cages settled on floating structures moored to the dock, are set up at  
150 three locations: the rainwaters site, the fairway area and the marsh. The rainwaters and careening sites  
151 floated around 0.8 meters under water surface. The marsh probe is set on a pole at 1 meter under the  
152 water surface. These three stations are set up at less than 1 meter from the scallops (Figure 2).

153 Salinity and turbidity are laboratory calibrated using  $12880\mu\text{s}\cdot\text{cm}^{-1}$  and 100 and 1000 NTU  
154 standard. Calibration constants are checked to be within the acceptable range mentioned in the user  
155 manual. Laboratory calibrations of turbidity sensors for SSC (Suspend Sediment Concentration) are  
156 performed in a 40L black bucket using wet sediments collected under the instruments. SSC are  
157 validated by filtration, using GF/C filters on field samples. Times-series data are processed using  
158 Matlab routines. Inter-calibration of probes are checked. No significant differences are noticed (paired  
159 T-student test with simultaneous field-measurements,  $p < 0.01$ ).

160 The ROX Optical Dissolved Oxygen sensors (6150 ROX probe) are calibrated using the one  
161 point « saturated water » method, mentioned in the « Calibration, Maintenance and Troubleshooting  
162 Tips » manual.

163 We carry out a post-deployment calibration on chlorophyll measurement using field  
164 samplings. Filters are analysed using a fluorometric method with acetone 90% (Fluorometer Turner  
165 TD 700), as proposed by Lorenz (1967).



166            **2.3 Trace element assessments**

167            Trace elements analyses are carried out according to previous studies (Milinkovitch et al.,  
168 2015; Breitwieser et al., 2017). Analyses of 14 trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni,  
169 Pb, Se, Sn, V, Zn) are performed using two measuring instruments: a Varian Vista-Pro ICP-OES and a  
170 Thermofisher Scientific XSeries 2 ICP-MS.

171            **2.4 Biomarker assessments**

172            All the biochemical assays (GST, Laccase, SOD, MDA) are performed using the same  
173 instrument: SAFAS Flx-xenius at the specific absorbance for each biomarker kind. The assays were  
174 carried out on the digestive glands of *M. varia* at the reference site (marsh) and the contaminated sites  
175 (outlet rainwaters and careening areas) at D0, 7 and 21 (D0, D7, D21, n = 10 per site and per date).  
176 Before statistical analysis, row data for GST, SOD and Laccase are expressed in U/mf of proteins.  
177 MDA row data concentrations are expressed in  $\mu\text{M}/\text{mg}$  of protein. Total protein concentrations are  
178 determined using the BCA Kit methodology (Bicinchononique Acid Kit, Sigma Aldrich). The BCA  
179 kit contains bovine serum albumin (BSA) as a standard and involves the reduction of alkaline  $\text{Cu}^{2+}$  by  
180 proteins (Smith et al., 1985) at absorbance 562 nm.

181            Moreover, another method to assess metallic pollution is used as additional data (Table S1).  
182 According to Mouneyrac (1998), metallothionein concentrations are determined in the digestive  
183 glands of scallops sampled at the same dates (D0, D07 and D21).

184            **Glutathion S-transferase (GST) assay**

185            Glutathion S-transferase (GST) specific activity plays an important role in xenobiotic  
186 detoxification. Total activity of GST (GST being a group of enzymes) is determined according to the  
187 kit method of Sigma (CS0410-1KT) (Breitwieser et al., 2017).

188            **Superoxide Dismutase (SOD) assay**

189            Superoxide dismutase (SOD) activity plays a key role in antioxidant response (Paoletti et al.  
190 1986).

191 *Malondialdehyde (MDA) assay*

192 Oxidative stress is assessed through lipid peroxidation by quantifying malondialdehyde  
193 (MDA), a chemical metabolite of cell lipid degradation. MDA concentration is measured using a  
194 commercially available MDA assay kit (Oxis International) (Milinkovitch et al. 2015).

195 *Laccase assay*

196 The phenoloxidase (PO) activity (Laccase) reveals bivalve's immune system alteration. Its  
197 enzymatic activity is contaminant sensitive (Luna-Acosta et al., 2010) and plays a crucial role in the  
198 immune defence mechanism of marine invertebrates.

199 *2.5 Statistical analysis*

200 Statistical analysis is performed using R software (R core team, 3.2.2 version). The survey is  
201 structured according to the dates (3 dates), the sites (3 sites), and the size of the samples for each sub-  
202 group (10 specimens only).

203 For all variables, homogeneity of variances is assessed using Fligner-Killeen tests. Then, the  
204 multivariate response of individuals at our three sampling sites and D0, D07, D21 is measured using a  
205 Multivariate Factorial Analysis. Indeed, the sample group is of 10 specimens. Each sample  
206 distribution reveals great heterogeneity for each variable. Therefore, it is unlikely to conduct a  
207 MANOVA, considering the number of trace elements (14), the number of morphological traits (3) and  
208 the number of biomarkers (4). In order to overcome this issue, all variables are divided into two  
209 sections: low and high, whether the value of the sample is under or above the median value of the 70  
210 individuals. This should also prevent from further issues due to abnormal values and non-normal  
211 distribution of variables inside each group of individuals. Normality is tested using a Shapiro-Wilk  
212 test. The normality hypothesis inside sub-groups (of 10 specimens) is rejected most of the time when  
213 examining the 21 variables. At D0 only 10 specimens are sampled, whereas 30 specimens are  
214 collected both at D7 and D21. Therefore, values of D0 individuals are weighted by 3 to balance the  
215 results between the 3 periods. Thus, all variables are recorded as being high (1) or low (0) for each

216 individual. Then, a qualitative multivariate analysis is conducted to underline relationships between  
217 variables (site, date, heavy metals, biomarkers and biometry parameters).

## 218 **3. Results**

### 219 ***3.1 Water physical parameters***

220 The water physical parameters are presented in Table 2. We perform daily averages to  
221 understand the general trends of the environmental conditions. The temperature trends range from  
222 16.8°C to 19.7°C and are representative of current weather conditions. In contrast, salinity and  
223 turbidity vary depending on the climatic conditions ranging from 27.61 to 33.19 PSU, and 0.004 to  
224 0.037 g/L. These measures are mainly due to rainfall bringing fresh water and sediment. This  
225 phenomenon is observed at the rainwater station at D21. Chlorophyll trends also differ very slightly at  
226 sites and dates, likely due to sunstroke. Dissolved oxygen rarely drops below 80% in surface water.  
227 100% saturations at the surface water occur during renewal of water bodies and in presence of wind.

### 228 ***3.2 Levels of trace elements***

229 Table 3 presents the results of the analyses on heavy metals performed in the digestive glands  
230 of the black scallop *M. varia*. These results are presented in µg/g of dry weight.

231 By comparing these 14 heavy metal values with the ones from previous studies in pectinids  
232 (Metian et al., 2008; Milinkovitch et al., 2015; Viarengo et al., 2000; Breitwieser et al., 2017), results  
233 reveal a lower contamination (in italic in the table 3) by Pb at D7 (-30%) at all sampling sites in  
234 comparison with the marsh ( $2.3 \pm 0.1$  µg/g of dry weight). In addition, there is a lower contamination  
235 (in italic in the Table 3) by Cd (-18%), Co (-28%), Mn (-30%), Pb (26.2%), Se (-23%), Zn (-14%),  
236 and Ag (-20%) at the rainwaters and careening sites at D21, in comparison to the reference site. In  
237 contrast, there is a higher contamination (in bold in the Table 3) by As (+7.5%), Cd (+14%), Cr  
238 (+125%), Cu (+423%), Fe (+ 18%), Mn (+18%), V (+62%) and Zn (+117%) at the rainwater or/and  
239 careening sites at D7 as well as a higher contamination (in bold in the table 3) by As (+12%), Cr, Cu  
240 (+54%) and Fe (+52%) at the same sites at D21.

241 **3.3 Biomarker responses**

242 The enzymatic assays presented in Figure 3 underline the state of health of marine bivalves.  
243 These are performed in order to target the capacities of detoxification of xenobiotics (GST) and to  
244 assess the struggle (SOD) against reactive oxygen species (ROS), the integrity of cell membranes, the  
245 involvement of lipid peroxidation (MDA), and the modulations of the immune system (Laccase).

246 The scatter plot in Figure 3 displays an easy-to-read, descriptive and multi-criteria analysis  
247 that compares results depending on the sampling dates and the caging sites of variegated scallops. All  
248 pollution biomarkers show significant modulations of their responses at various dates and the two  
249 intra-harbour sites.

250 The plot in Figure 3 presents GST distributions. There are no significant differences. Only  
251 trends of mean specific activities seem to increase over time and are higher at the marsh site ( $0.75 \pm$   
252  $0.2$  UI/mg of proteins at D07,  $1 \pm 0.2$  UI/mg of proteins at D21) than at the rainwater ( $0.66 \pm 0.1$   
253 UI/mg of proteins at D07,  $0.85 \pm 0.1$  UI/mg of proteins at D21), or the careening areas ( $0.7 \pm 0.1$   
254 UI/mg of proteins at D07,  $0.94 \pm 0.1$  UI/mg of proteins at D21). The non-parametric test of Fligner-  
255 Killeen for homogeneity of variance with a p-value of 0.5817 confirms that we can compare both  
256 dates and sites for GST.

257 In contrast, MDA homogeneity of variance test fails either for date (p-value = 0.0388), site (p-  
258 value = 0.0013), and both date and site (p-value = 0.0002, < 0.05). In fact, at the rainwater site at D21,  
259 the large distribution is characterised by a high heterogeneity of MDA concentration ( $16.1 \pm 13.4$   
260  $\mu\text{M}/\text{mg}$  of proteins), which is not influenced by dates or sites.

261 The analysis of the Laccase activity at the three sites and sampling dates reveals  
262 discrimination at all sites at D21. The non-parametric test of Fligner-Killeen for homogeneity of  
263 variance confirms (p-value = 0.4522) the analysis. Whereas the behaviour of the Laccase enzyme is  
264 similar at D0 and D7, and stable for all sites (average of  $4.5 \pm 1$  UI/mg of proteins), there is an overall  
265 increase at D21. However, at D21, Laccase is lower at the careening site ( $7.5 \pm 1.1$  UI/mg of proteins)  
266 than at the other sites ( $10.3 \pm 2$  UI/mg of proteins).

267           Superoxide Dismutase activity is compared for both sites and dates, as shown by the non-  
268 parametric test of Fligner-Killeen for homogeneity of variance (p-value = 0.2022). The rainwaters and  
269 careening sites have similar patterns: they increase slowly between D7 and D21, although they remain  
270 lower than the levels at the marsh site at D0 ( $57 \pm 8$  UI/mg of proteins) and D7 ( $72 \pm 12$  UI/mg of  
271 proteins). The marsh site highlights an irregular pattern: starting with high levels at the beginning (D0  
272 and D7), they increase drastically before dropping at D21 ( $23 \pm 11$  UI/mg of proteins). The activity  
273 levels at D21 are lower than the ones of the rainwaters ( $40 \pm 21$  UI/mg of proteins) and careening sites  
274 ( $30 \pm 11$  UI/mg of proteins).

275           Moreover, metallothionein concentrations presented in Table S1 show a significantly higher  
276 response and a specific inorganic contamination at the careening and rainwaters areas, in comparison  
277 to the marsh site at D7.

### 278           ***3.4 Factor analysis to underline relationships between biomarker responses and trace*** 279           ***element assessments***

280           Figure 4 displays an easy-to-read, descriptive and multi-criteria analysis that compares results of  
281 the qualitative analysis used (“low signal” or “high signal”), the sampling dates and the various sites.  
282 The analysis focuses on the two first components, since the others do not bring further details (Figure  
283 S1). The first two axes explain 36.92% of the variance, which is enough to perform a qualitative factor  
284 analysis.

285           Looking at axis 1 (horizontal dimension), variables above the 0.5 contribution value (Figure S2 in  
286 supplementary data) indicate a high concentration of Pb and Ag, and a low concentration of Cu, Sn  
287 and As linked to strong SOD and low Laccase activities. Variables under the -0.5-contribution value  
288 show a high concentration of Cu, Cr, Mn, Sn, and As linked to a strong Laccase and GST activities,  
289 but a low SOD activity.

290           Looking at axis 2 (vertical dimension), variables above the 0.5 value shows the antagonistic effect  
291 of low concentration (for *high.0*) of Ni, Co, Pb, Mn elements against the effect of high concentration  
292 (for *high.1*) of Pb, Fe, Se, Ni, Zn, Co, and Mn.

293 Thus, individuals at the right of axis 1 have a strong SOD activity but a low Laccase activity. They  
294 seem to be less exposed to heavy metal elements than those on the left side of axis 1. Axis 1 reveals  
295 the effect of Cu, Sn and As on enzymatic activities (SOD, Laccase and GST), as a major interaction of  
296 the state of health of scallops. Furthermore, GST and SOD seem to have antagonist effects. Axis 2  
297 underlines a separation between high concentrations of heavy metals such as Mn, Co, Zn, Ni, Se, Fe,  
298 and Pb and low concentrations of Mn, Ni, Co, Pb. It highlights the preponderant concentration of Mn,  
299 Ni and Pb in scallops (individuals having a high concentration in heavy metals are shown at the  
300 bottom of the Figure 4).

301 In order to check whether the site and date factors (temporal dimension) influence the  
302 specimen's repartition on the plan defined by axes 1 and 2, we have drawn confidence ellipses on the  
303 plot of Figure 4. A label and a distinct colour are assigned for each group or individual. Groups are  
304 divided as followed: D0, D7 and D21 at the marsh, D0, D7 and D21 at the careening area, and D0, D7  
305 and D21 at the rainwaters site. Harbour sites (careening and rainwaters) have similar evolution for  
306 each date (D7 and D21), in comparison to the marsh groups, which are merely located at the right of  
307 axis 1. Over time (from D0 to D21), a movement towards the bottom of the axis 2 is common to all  
308 groups, which is symptomatic of an increasing concentration of Pb, Fe, Se, Ni, Zn, Co, and Mn trace  
309 elements. Equally, a movement of all groups towards the left of the axis 1 can be observed, due to a  
310 higher concentration of Fe, As, Sn, Mn, Cr, Cu trace elements conjointly with stronger GST and  
311 Laccase activities, and a lower SOD signal.

## 312 **4. Discussion**

### 313 ***4.1 Study method***

314 So far, few caging multi-biomarker studies (biomarkers of effect and exposure) have been  
315 carried out in France using other molluscs such as the freshwater mussel *Unio tumidus* (Charissou et  
316 al., 2004), and the marine mussel *Mytilus* spp. (Lacroix et al., 2017). Caged bivalves could be  
317 successfully used as sentinel animals of contamination events in marine ecosystems. Some data show  
318 that the uptake and elimination of metals were evaluated in different tissues of the mussel *Pinna*

319 *nobilis* L. transplanted to harbour's area of Tunisia (Jebali et al., 2014). Moreover, mussels of the  
320 specie *Mytilus galloprovincialis* could also reveal high levels of contaminants in bioactive monitoring  
321 experiments conducted in Spain (De los Rios et al., 2012; Marigomez et al., 2013; De los Rios et al.,  
322 2018).

323 The black scallop *M. varia* is located in the subtidal zone, unlike oysters and mussels that live  
324 higher in the intertidal zone. Unlike the last two species, the black scallop and its digestive glands are  
325 used as tools revealing the environmental quality of the port area (Breitwieser et al., 2017).

326 One of the goals of this study is to assess the feasibility of a caging experiment on marine  
327 bivalves of similar backgrounds in order to compare the environmental quality between intra-harbour  
328 sites (potentially contaminated environments by various chemical compounds) and a less  
329 contaminated area that had been rarely done before on variegated scallop (*M. varia*).

330 To this end, various precautions are taken: the choice of a calm and safe zone for the  
331 installation of the cages, the types of cages (dimensions and meshes adapted to the species) and the  
332 choice of the species, adapted to the condition's environmental aspects of the study areas. The  
333 shellfish from the Tinduff Hatchery are 18 months old and already acclimated to caging experiment  
334 conditions. In addition, the dissection of living bivalves is carried out on site to prevent from  
335 disruptions of the enzymatic activities.

336 Moreover, even if each individual is considered as a replicate of the study, we duplicate cages,  
337 fixed to a floating structure on each site, to accentuate the contact with trapped pollutants in the  
338 sediment. The cages are risen to the surface at each sampling date – D0, D07, D21 – (as exposure time  
339 is necessary for the bioaccumulation of bivalves). Our experimental design inspired of the study of  
340 Charissou (2004) is agreeing with the recent work which aimed to study impacts of dredging  
341 operations on biomarkers responses of caged bivalves (Moreira et al., 2019).

342 **4.2 Sensitivity and relevance of parameters**

343 The chemical composition of the water samples does not enable to classify the sites as  
344 mentioned by Charissou (2004). All the parameters suggest normal water quality based on values  
345 measured at each harbour site at D0.

346 Biomonitoring of variegated scallops allows putting forward a global classification of the three  
347 sites. In decreasing order of the biological quality, the classification is: careening<rainwaters<marsh.  
348 The marsh is considered as the best site, as only a few heavy metals are significantly higher, when  
349 compared to the other sites.

350 Many scientific studies underline that higher heavy metals values in bivalve organisms reveal  
351 an exposition to metal compounds and, thus, a risk of having toxic effects (Bouchard, 2018). At D21,  
352 our results show higher concentrations of As, Cr, Cu, Fe and Sn at the careening area, when compared  
353 to the marsh. Moreover, As, Cu, Fe concentrations are higher at the rainwaters area than at the marsh.  
354 Our results support the hypothesis of the World Health Organization and the potential ecological risk  
355 of environmental pollutants. It states that ambient environmental contamination is caused by a  
356 combination of agents from public road, roofing panels, metal adhesives, pigments, metal industry,  
357 and antifouling paint (Pourabadehei and Mulligan, 2016; Ranjbar Jafarabadi et al., 2017; Chen et al.,  
358 2017; Jahan and Strezov, 2018; Gargouri et al., 2018; Kim et al., 2018; Tunca et al., 2018).

359 After 21 days, concentrations of bioaccumulation in *in situ* scallops are similar to previous  
360 studies performed at the same location. Moreover, the biomarker response of transplanted bivalves  
361 matched the results of *in situ* bivalves obtained from a previous study (Breitwieser et al., 2017).  
362 Overall, this research revealed a SOD decrease, Laccase and GST stimulations, and higher  
363 concentrations in Cu, Fe, As, Co, Mn, Zn, Sn in the digestive glands. This is an interesting criterion to  
364 consider for future studies using the specie *M. varia*. Nonetheless, this finding does not explain the  
365 toxicology of heavy metals, which depends on molecular shapes. New methods for the analysis of  
366 toxicology induced by heavy metals could be used, such as the quantum dot method used for cadmium



367 (Jimeno-Romero et al., 2019) where the solubility of particles would be partly the cause toxicity  
368 generated in bivalves.

369 In addition to heavy metal assessments, it will be relevant to write down all the contaminants  
370 of the harbour of La Rochelle. This list could include pesticides already identified in coastal waters, as  
371 biocides (diuron, triazine) used in antifouling paint of ship and small boat, pharmaceutical residues  
372 and other micro-pollutants (micro-plastics, phtalates), which are all potentially dangerous for the  
373 health of organisms, and, thus, to consumers at the end of the food chain.

374 Multi-criteria analysis (Figure 4) is carried out. We compare the inorganic contaminant,  
375 molecular biomarker and quality qualitative analyses (“low signal=high.0” or “high signal=high.1”) as  
376 well as the sampling dates and the various sites. Pollutant qualitative results (Pb, Fe, Se, Ni, Zn, Co,  
377 Mn, Cr, Sn, As) in the digestive gland of this species are positively correlated with biomarker of  
378 responses (GST and Laccase) and negatively correlated with the SOD biomarker. The stability of the  
379 MDA results reveals the efficacy of anti-oxidants and lipid peroxidation in variegated scallops, as  
380 already found in juvenile bivalves (Roméo et al., 2008). Moreover, several natural abiotic factors  
381 (turbidity, chlorophyll a, oxygen level, temperature, salinity) could also explain the modulation of  
382 oxidative stress, although no significant modulation is observed. Thus, the digestive glands of the  
383 variegated scallop are relevant organs to assess the environmental quality of the harbour (Triebkorn et  
384 al., 2001) as biomarker results reveal similar modulations to previous studies using this specie  
385 (Milinkovitch et al., 2015; Breitwieser et al., 2016; Breitwieser et al., 2017). Indeed, three biomarkers  
386 of exposure (Laccase, SOD and metallothioneins) show significant responses and the GST biomarker  
387 highlight an activity trend that displays environmental stress. These results suggest that the oxidative  
388 stress and reactive oxygen species neutralisation could be due to a mixture of contaminants (e.g  
389 pesticides, hydrocarbons, heavy metals) from wastes of watershed and urban activities (Luschak,  
390 2011).

391 These molecular biomarkers of exposure are relevant and efficient tools to use for the harbour  
392 authorities. Overall the study demonstrated that differences in chemical bioaccumulation and

393 biomarker responses, and confirm results of Brooks et al., 2015 which explain the importance of  
394 identifying the pertinent species when using bivalve in biological effects studies. In addition, new  
395 easy, rapid and inexpensive methods could be implemented. For instance, energetic metabolism with  
396 ATP assay could be linked to behavioural parameters (reproduction, growth). The study of energy  
397 allocation mechanisms at the biomolecular level up to the cellular level could bring a better knowledge  
398 of the responses of *M. varia*. Indeed, the energy cost of environmental stress may decrease the amount  
399 of energy available to maintain homeostasis and, therefore, reduce the fitness of individuals as  
400 highlighted in sea fish and mussel species (Lauwrence et al., 2003; Donaghy et al., 2016). The  
401 majority of energy would be allocated to the development of defences, which could lead to the  
402 increase in tolerance of marine species to environmental chemical contamination. Energy metabolism  
403 therefore remains a relevant pathway to investigate in order to understand how populations maintain  
404 themselves in the environment, particularly with abundance fluctuations between both species. It  
405 could be complementary to plan also to consider current perspectives and future directions of three  
406 main omics (transcriptomics, proteomics and metabolomics) with regards to identify, characterize and  
407 determine functional aspects across biological processes to better understand bivalve physiology,  
408 especially with regards to immune defences to environmental stresses (Fasulo et al., 2012b; Gouveia  
409 et al., 2019; Nguyen et al., 2019). Finally, it seems to be urge (1) to compare the concentration of trace  
410 elements found in bivalve *M. varia* collected from the La Rochelle Marina with the xenobiotics data  
411 determined with those reported in other French port's areas and (2) to participate to the enrichment of  
412 the data available on the potential risks to human health associated with the consumption of marine  
413 bivalve (Marengo et al., 2018).

## 414 **Conclusion**

415 This field study attests that scallops, particularly the digestive glands, are effective tools to  
416 assess environmental port's quality. Biochemical parameters could represent reliable biomarkers for  
417 the assessment of environmental pollution on *M. varia* as biological biomonitoring model. In addition,  
418 for the first time, the research reveals a rapid bioaccumulation of heavy metals in this specie as well as  
419 a similar toxicity to previous studies performed *in situ* in the marina of La Rochelle. Further

420 investigations are needed to evaluate the environmental impacts of environmental (biotic and abiotic)  
421 factors using multi-biomarker approaches to confirm such point of view.

422 Coastal waters collect storm waters containing substances, such as heavy metals and  
423 pesticides, which have negative impacts on the water quality. It could be interesting to explore some  
424 differences in histological microbiological and biomarker responses of scallops were site-dependent.  
425 In addition, dilution caused by tides, currents and rainfall have adverse effects on water. Thus, it is  
426 essential to develop low-cost and transposable approaches for monitoring the water quality in similar  
427 areas. Finally, we propose the use of scallops as indicator species for monitoring of diffuse pollution  
428 in the marine environment. We could consider the formation of European workgroup of marine  
429 chemical contamination in bivalve to develop an internationally accepted protocol to conduct  
430 harbour's active biomonitoring and collect preliminary data comparing coastal scallops from around  
431 the European port's area.

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
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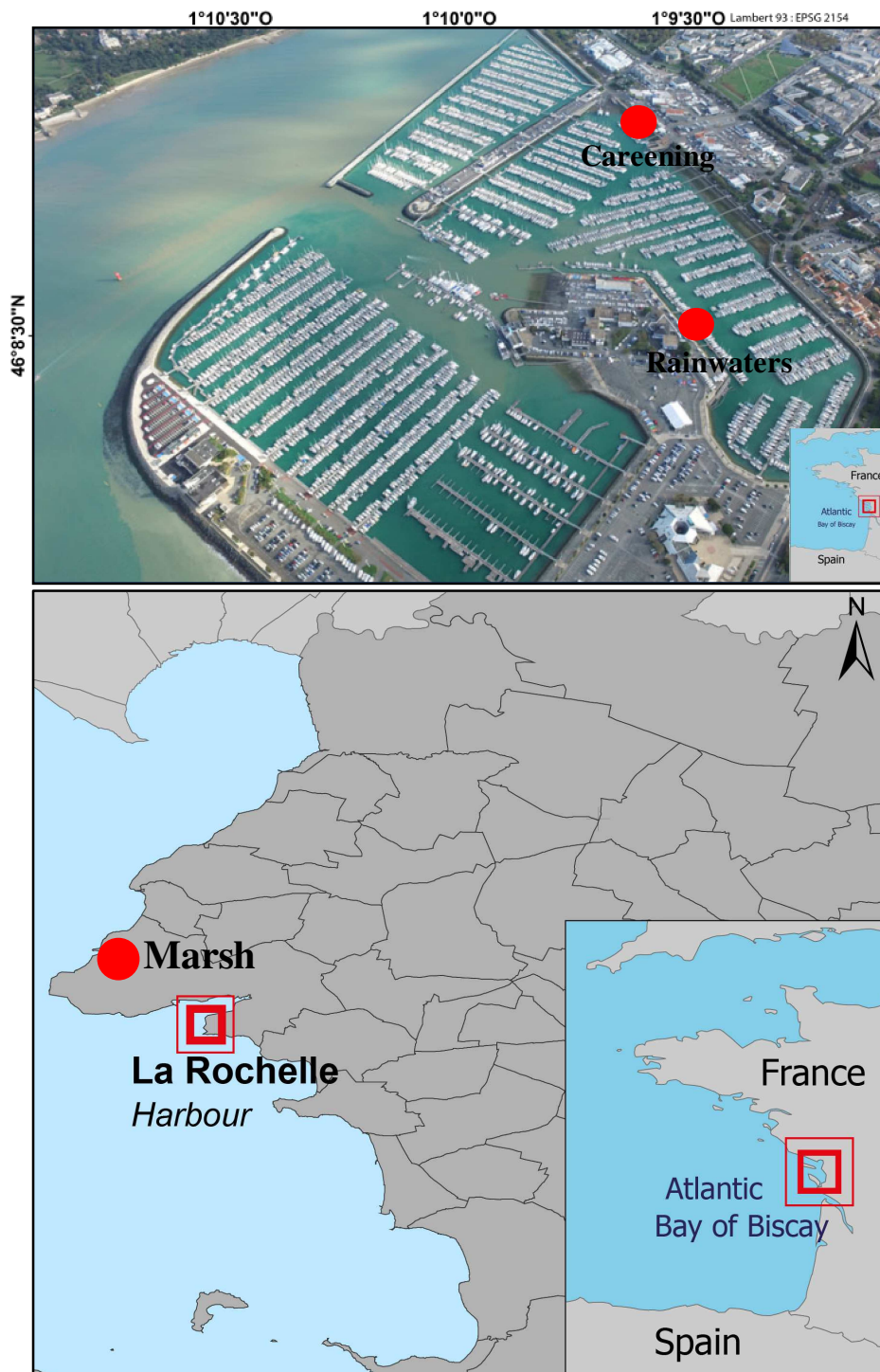
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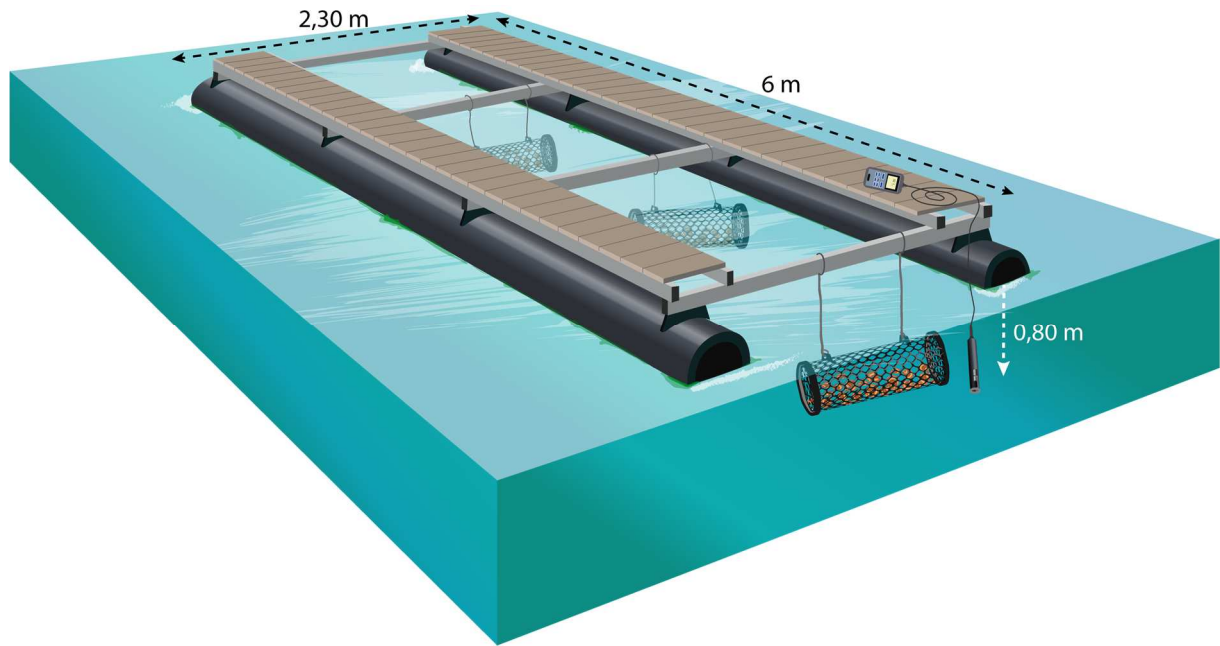
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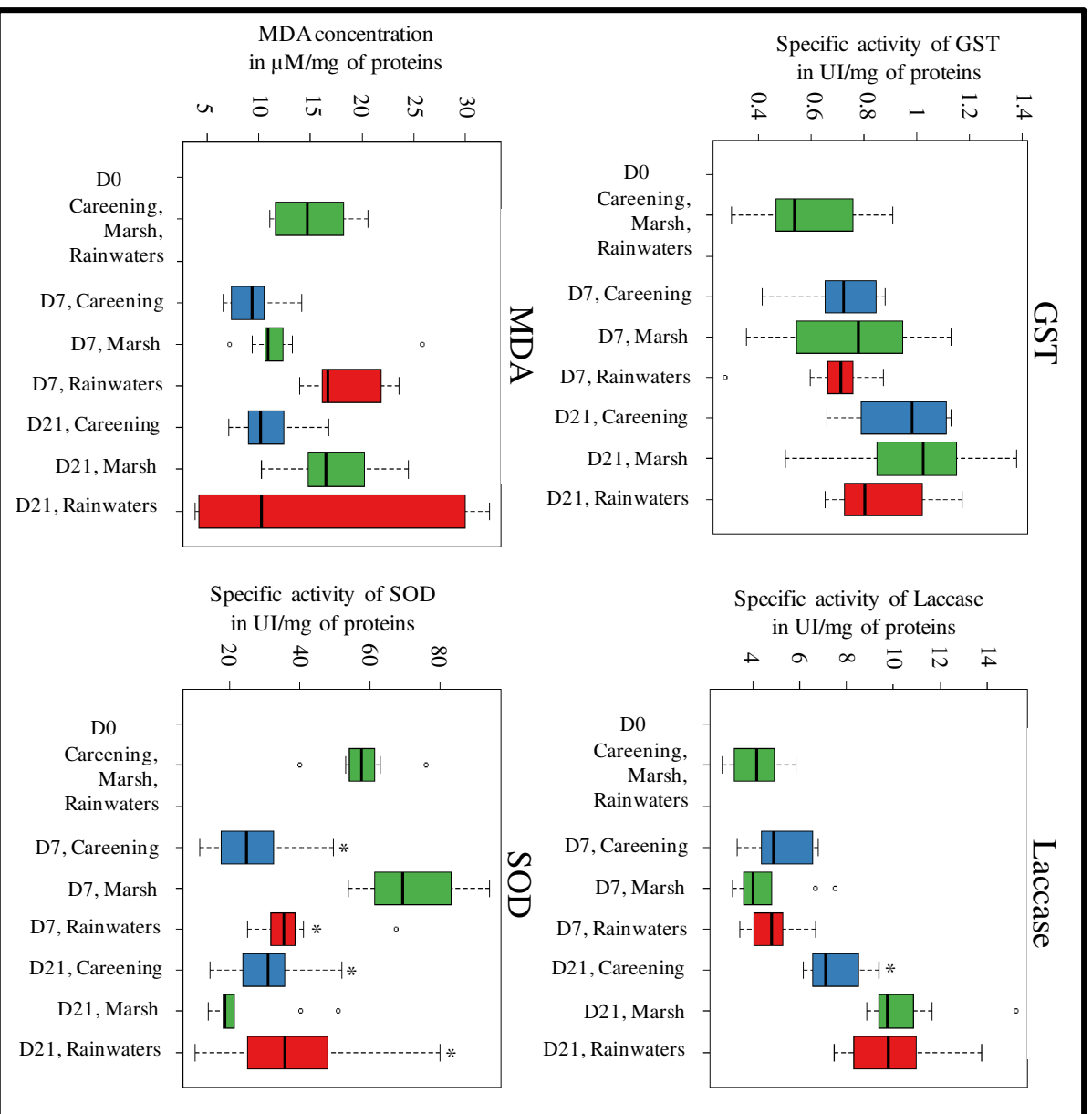
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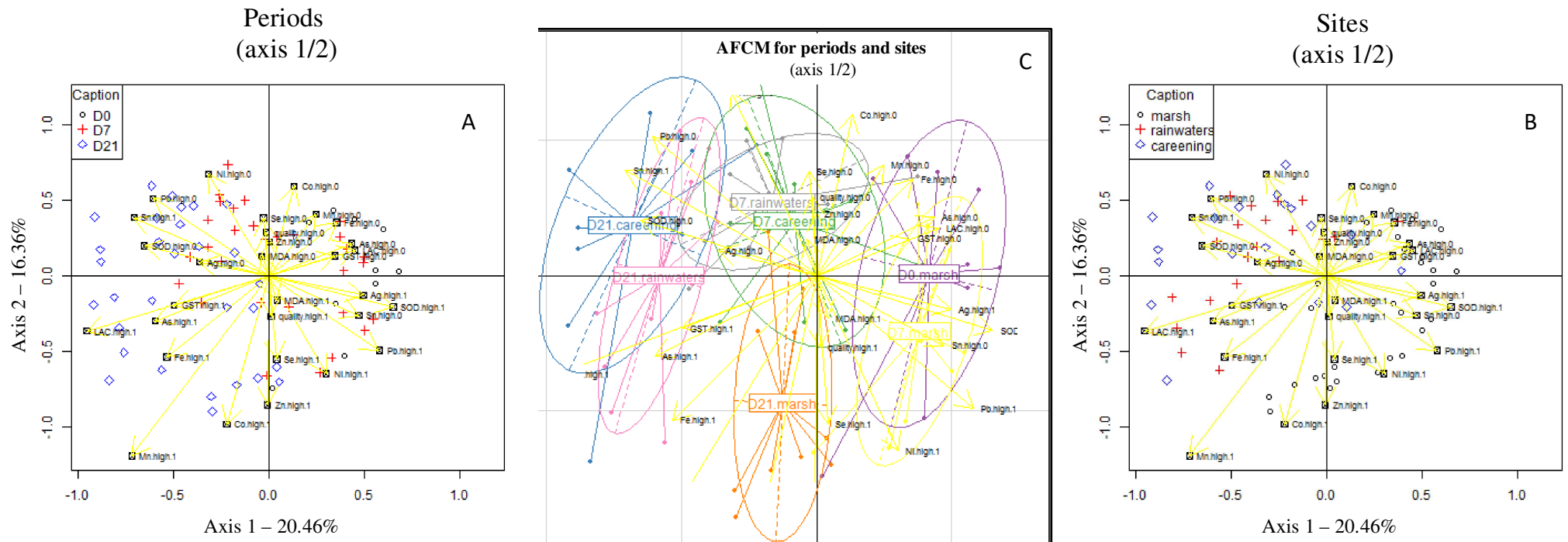
**Figure 1:** Map of France including the locations of the study sites. The marsh is the reference site (less-contaminated). The two impacted areas are the careening and the rainwaters sites, located in the harbour of La Rochelle.



**Figure 2:** Illustration of the monitoring station with floating structures for the three locations: the rainwaters site, the fairway area and the marsh. (Source: Thierry GUYOT (LIENSs)).



**Figure 3:** Boxplots showing biomarker results in the digestive glands of *M. varia* at the reference site (marsh) and the contaminated sites (outlet rainwaters and careening areas) at D0, 07 and 21 (D0, D07, D21, n = 10 per site and per date). Significant differences between impacted sites and the marsh (control site) for each date are indicated using asterisks: \* $p < 0.05$ ; \*\*\* $p < 0.001$ .



**Figure 4:** Multiple factor analysis plots indicating individual responses at the three sampling sites (represented in different colours) at D0, D07 and D21 as well as the qualitative trace metal variables explaining variations in the individuals x biomarkers matrix depending on periods or sites. (A: results by period, B: results by site, C: results by period and sites). The first two axes reveal a 36.92% of variance in data (20.46% and 16.36%, respectively). MDA: MDA contents, SOD: SOD activity, GST: GST activity, LAC: Laccase activity.

**Table 1:** Number, length, width and thickness of scallops and weight of digestive gland sampled at the three sites (marsh, careening and rainwaters) at D0, D07 and D21.

	Marsh			Careening			Rainwaters		
	D0	D07	D21	D0	D07	D21	D0	D07	D21
<b>Number</b>	10	-	-	10	10	10	10	10	10
<b>Weight (mg)</b>	432.62	396.90	413.70	-	383.60	461.20	-	365.20	421.40
<b>Length (mm)</b>	41.70	41.20	44.20	-	44.80	43.60	-	39.94	42.40
<b>Width (mm)</b>	37.70	35.30	38.00	-	39.60	37.90	-	34.11	39.80
<b>Thickness (mm)</b>	14.50	14.15	15.80	-	15.30	14.00	-	12.78	16.00

**Table 2:** Physicochemical parameters at the three sampling sites: careening, rainwaters and marsh (reference site) areas. Temperature (Temp) is in degrees Celsius, salinity (Sal) in Practical Salinity Unit (PSU), turbidity of sediments in Sed (g/L), chlorophyll a (Chla) in µg/L, dissolved oxygen (O<sup>2</sup>) in percentage of saturation, and N.d indicates No data.

	Rainwaters			Careening			Marsh		
	D0	D07	D21	D0	D07	D21	D0	D07	D21
<b>Temp (°C)</b>	16.80	16.95	18.92	17.00	16.82	19.01	N.d	N.d	19.70
<b>Sal (PSU)</b>	30.54	31.61	27.61	31.96	31.94	32.00	N.d	N.d	33.19
<b>Sed (g/L)</b>	0.004	0.008	0.037	0.005	0.015	0.009	N.d	N.d	0.012
<b>Chla (µg/L)</b>	3.62	2.49	3.67	2.68	2.58	3.21	N.d	N.d	N.d
<b>O<sup>2</sup> (%)</b>	87.70	81.82	100	100	94.74	100	N.d	N.d	N.d

**Table 3:** Trace element concentrations ( $\mu\text{g/g}$  of dry weight) in the digestive glands of *M. varia* ( $n = 10$  per site for each date) at the three sampling areas: the marsh (reference site), the careening and rainwaters areas.

Stars (\*) above values in **bold indicate significantly higher difference** where  $p < 0.05$  between the marsh and the harbour sites at each date and for each heavy metal. Stars (\*) above values in *italic indicate significantly lower difference* where  $p < 0.05$  between the marsh and the harbour sites at each date and for each heavy metal.

Dates	Sites	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	V	Zn	Ag	Sn
D0	Marsh	18.32 $\pm$ 0.9	32.32 $\pm$ 2	0.7 $\pm$ 0.04	1.3 $\pm$ 0.09	21.28 $\pm$ 2.5	566 $\pm$ 71	14.28 $\pm$ 2	2.08 $\pm$ 0.1	2.5 $\pm$ 0.2	9.1 $\pm$ 0.6	5.5 $\pm$ 0.6	157 $\pm$ 53	4.7 $\pm$ 0.7	0,02
D0	Careening	18.32 $\pm$ 0.9	32.32 $\pm$ 2	0.7 $\pm$ 0.04	1.3 $\pm$ 0.09	21.28 $\pm$ 2.5	566 $\pm$ 71	14.28 $\pm$ 2	2.08 $\pm$ 0.1	2.5 $\pm$ 0.2	9.1 $\pm$ 0.6	5.5 $\pm$ 0.6	157 $\pm$ 53	4.7 $\pm$ 0.7	0,02
D0	Rainwaters	18.32 $\pm$ 0.9	32.32 $\pm$ 2	0.7 $\pm$ 0.04	1.3 $\pm$ 0.09	21.28 $\pm$ 2.5	566 $\pm$ 71	14.28 $\pm$ 2	2.08 $\pm$ 0.1	2.5 $\pm$ 0.2	9.1 $\pm$ 0.6	5.5 $\pm$ 0.6	157 $\pm$ 53	4.7 $\pm$ 0.7	0,02
D07	Marsh	18.77 $\pm$ 0.8	29.95 $\pm$ 2.1	0.80 $\pm$ 0.05	1.2 $\pm$ 0.09	21.30 $\pm$ 2.1	488 $\pm$ 57	22.37 $\pm$ 3.4	2.3 $\pm$ 0.1	2.3 $\pm$ 0.1	11.35 $\pm$ 1	4.5 $\pm$ 0.6	157 $\pm$ 33	4.3 $\pm$ 0.8	0,01
D07	Careening	<b>20.15 <math>\pm</math> 1.5*</b>	<b>33.46 <math>\pm</math> 2.9*</b>	0.78 $\pm$ 0.05	1.5 $\pm$ 0.08	<b>109 <math>\pm</math> 11*</b>	<b>570 <math>\pm</math> 68*</b>	<b>26.15 <math>\pm</math> 8.2*</b>	2 $\pm$ 0.1	<i>1.7 <math>\pm</math> 0.1*</i>	9.6 $\pm$ 0.9	<b>7.3 <math>\pm</math> 0.4*</b>	<b>342 <math>\pm</math> 157*</b>	4.2 $\pm$ 0.7	0,06
D07	Rainwaters	19 $\pm$ 0.8	29.10 $\pm$ 1.2	0.76 $\pm$ 0.01	<b>2.7 <math>\pm</math> 0.7*</b>	<b>112 <math>\pm</math> 18*</b>	<b>580 <math>\pm</math> 64*</b>	19.81 $\pm$ 2.1	2 $\pm$ 0.1	<i>1.5 <math>\pm</math> 0.07*</i>	9.1 $\pm$ 0.8	<b>6.8 <math>\pm</math> 0.8*</b>	159 $\pm$ 63	<b>5.2 <math>\pm</math> 1.7*</b>	0,07
D21	Marsh	18.76 $\pm$ 1.1	33.63 $\pm$ 3.2	1.1 $\pm$ 0.05	1.3 $\pm$ 0.08	18.77 $\pm$ 1.6	506 $\pm$ 68	40.26 $\pm$ 6.1	2.3 $\pm$ 0.1	2.1 $\pm$ 0.2	10.75 $\pm$ 0.5	5.5 $\pm$ 0.9	248 $\pm$ 100	4.4 $\pm$ 0.6	0,01
D21	Careening	<b>21.17 <math>\pm</math> 0.4*</b>	32.70 $\pm$ 3.2	<i>0,83 <math>\pm</math> 0.07*</i>	<b>2 <math>\pm</math> 0.3*</b>	<b>287 <math>\pm</math> 48*</b>	<b>879 <math>\pm</math> 229*</b>	<i>28.32 <math>\pm</math> 4.6*</i>	1.8 $\pm$ 0.2	<i>1.7 <math>\pm</math> 0.3*</i>	<i>8.3 <math>\pm</math> 0.3*</i>	5,95 $\pm$ 0.6	<i>213 <math>\pm</math> 48*</i>	<i>3.5 <math>\pm</math> 0.4*</i>	<b>0,14*</b>
D21	Rainwaters	<b>22.02 <math>\pm</math> 0.9*</b>	<i>27.47 <math>\pm</math> 2.4*</i>	<i>0,75 <math>\pm</math> 0.05*</i>	1.6 $\pm$ 0.1	<b>353 <math>\pm</math> 38*</b>	<b>666 <math>\pm</math> 90*</b>	<i>28 <math>\pm</math> 4.5*</i>	1.4 $\pm$ 0.1	<i>1.4 <math>\pm</math> 0.2*</i>	9.7 $\pm$ 0.5	5.2 $\pm$ 0.5	235 $\pm$ 74	<i>3.5 <math>\pm</math> 0.8*</i>	0,07