

1           **Title**

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4           What is the relationship between the bioaccumulation of chemical contaminants in the  
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6           variegated scallop *Mimachlamys varia* and its health status? A study carried out on the French  
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8           Atlantic coast using the Path ComDim model.  
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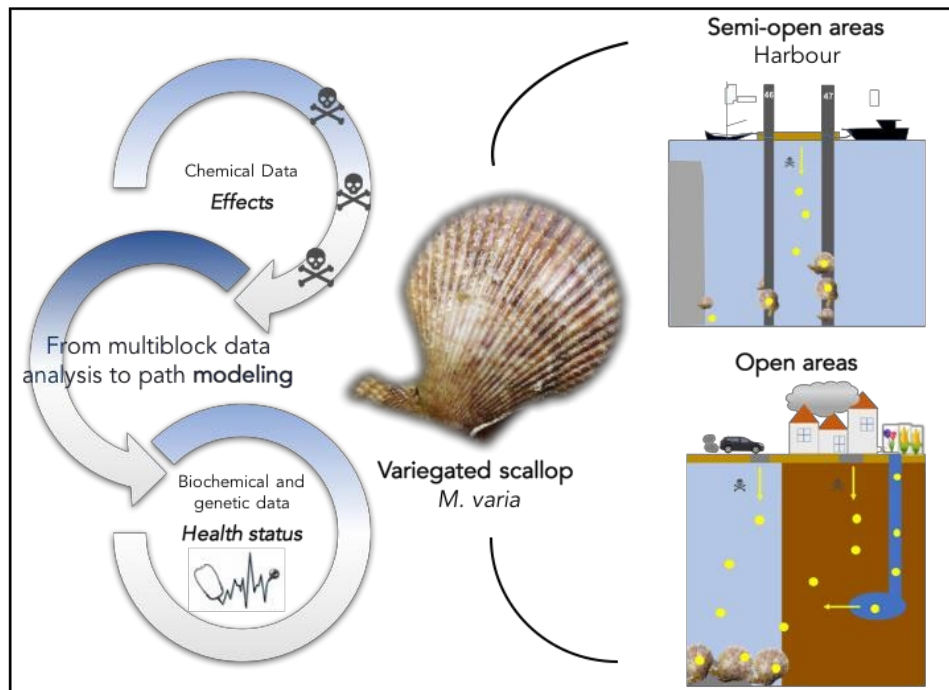
## Abstract

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Increasing activity along the French Atlantic coast has led to chronic pollution with, in particular, mixtures of contaminants such as hydrocarbons, phytosanitary products, PCBs and heavy metals. Based on previous research, pollution biomarkers were used in this study as they can indicate health status when monitoring the impact of pollutants on coastal species such as the marine bivalve *Mimachlamys varia*. Mollusc bivalves were sampled in March 2016, in open and semi-open areas (a harbour zone), from thirteen sites which differed in terms of their level of pollution, and were located along the Atlantic coast from Brittany down to the Nouvelle-Aquitaine region. First, analyses of heavy metals and organic contaminants (e.g. pesticides, polycyclic aromatic hydrocarbons, polychlorobiphenyl) in the digestive gland of bivalves were performed. Second, biochemical assays were used to study defence biomarkers: oxidative stress with Superoxide Dismutase (SOD), detoxification of organic compounds with Glutathione-S Transferase (GST), lipid peroxidation with Malondialdehyde (MDA), and immune processes with Laccase. In addition to the biochemical assays, a genetic approach was used to measure genetic diversity (haplotype and nucleotide diversity) at each site. Biomarker assays and genetic diversity were correlated with the chemical contaminants in bivalves using the Path-ComDim statistical model. Our results showed specific correlations between biochemical assays in the digestive glands with heavy metal contaminants, and between genetic diversity and organic pollution. Blocks of responses were analysed for correlations in order to develop standardized tools and guidelines that could improve our understanding of the short-term and long-term impact of contaminants on physiological parameters.

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**Keywords:** *Mimachlamys varia*, chronic chemical contamination, Atlantic coast, genetic diversity, biochemical assays, Path-ComDim model



Integrative approach undertaken in this study to assess effects of a chronic chemical contamination on natural populations of the marine bivalve *Mimachlamys varia*.  
Photo credit: Thierry Guyot.

## Highlights

- *Mimachlamys varia* was shown to be a useful bioindicator for environment quality assessment
- Scallops were collected from 13 areas along the French Atlantic coast, contaminated to different degrees with organic compounds and heavy metals
- Relationships between blocks of variables (contaminants, biomarkers of pollution, genetic diversity) were demonstrated with the Path-ComDim model
- The health of marine bivalves is modulated by polymetallic pollution
- Genetic diversity was negatively correlated to organic contamination

## 1 1. Introduction

2 Run-off water containing various chemical contaminants accumulates on the coast.

3 The degree of long-term chemical contamination of coastal waters may vary depending on the  
4 effects of dilution as a result of tides, currents and climate variation. Since the 1970s  
5 (Convention for the Prevention of Marine Pollution from Land-Based Sources, 1974) there  
6 has been a growing awareness of the disastrous consequences of chemical contaminants on  
7 our health and on the well-being of the environment.

8 Aside from the cross-disciplinary policies on issues related to the coast and the maritime  
9 economy, framed by the French National Strategy for the Sea and the Coast and that was  
10 introduced in 2016 (by the Minister for the Environment, Energy and the Sea in August  
11 2016), the regulations concerning the quality of coastal waters are important both at the  
12 national and the European Community level. The regulations pertaining to water quality  
13 define the principles that must be adhered to by European Union member states in order to  
14 achieve a good ecological water status by 2020. These principles are defined in the Marine  
15 Strategy Framework Directive (MSFD), the Water Framework Directive (WFD), in measures  
16 taken by the farming sector (better management of fertilisation, the nitrate directive, the *plan*  
17 *Ecophyto*), and measures taken at the level of catchment areas. The aim is to reduce the use of  
18 phytosanitary, heavy metal, polychlorobiphenyl and hydrocarbon products and to help the  
19 local stakeholders, farmers and port managers to improve the quality of the water.

20 In this context, the aim of the present study was to assess the health of marine bivalves, which  
21 are excellent sentinel organisms for measuring the quality of coastal environments (Grosell  
22 and Walsh, 2006). Additionally, bivalves such as the variegated scallop *Mimachlamys varia*  
23 are commercialised along the French coast. Initial *in situ* ecotoxicological studies were  
24 carried out by our laboratory to assess the presence of pollutants in these organisms  
25 (biomarkers of exposure) and/or to measure the impact of pollutants (biomarkers of effects)

26 on this species, using both biochemical markers and genetic diversity measurements  
27 (Bustamante et al., 2005; Milinkovitch et al., 2015; Breitwieser et al., 2016, submitted-a).  
28 Here, a biomonitoring study was performed on 13 sites in the Nouvelle-Aquitaine and  
29 Brittany regions in March 2016 (Figure 1). Antioxidant enzyme activity (SuperOxyde  
30 Dismutase biomarker), detoxication enzyme activity (Glutathion S-transferase biomarker),  
31 lipid peroxidation (MalonDiAldehyde biomarker) and immunomodulation (Laccase  
32 biomarker) were used as pollution markers. Malondialdehyde and SOD form part of the  
33 antioxidant defences and have been used to show the impact of exposure to metals and a wide  
34 range of organic compounds in the environment (Amiard-Triquet et al., 2013). Glutathione S-  
35 transferases are involved in the phase II detoxification of organic contaminants, and also play  
36 a protective role in oxidative stress by catalysing a selenium-dependent glutathione  
37 peroxidase (Tappel et al., 1982; Prohaska, 1980). Lipid peroxidation in the MDA assay  
38 indicates damage to cellular membrane lipids caused by ROS and is useful for assessing  
39 exposure to and the effects of pollutants in marine species (Sureda et al., 2011; Capo et al.,  
40 2015). Moreover, in bivalves such as oysters, mussels and variegated scallops, there is a  
41 depletion of key enzymes such as laccase, which is involved in the modulation of the immune  
42 system in the bivalve in the presence of different kinds of contaminants (Luna-Acosta et al.,  
43 2010, 2011; Milinkovitch et al., 2015; Breitwieser et al., 2016). In addition, previous studies  
44 on other aquatic organisms have shown that chemical contamination can cause genetic  
45 damage at the molecular level and can affect population genetic diversity (e.g. Guttman,  
46 1994; Gardeström et al., 2008). A decrease in genetic variability can result from a reduction in  
47 population size due to an increased mortality rate and/or reduced fertility, and from natural  
48 selection of individuals with more resistant genotypes (van Straalen et al., 2002; Nowak et al.,  
49 2009; Bickham, 2011; Brady et al., 2017). As a result, investigating the genetic effects of

50 chemical contamination at the population level has been recognized as an important aspect in  
51 impact assessment (Bickham et al., 2000).

52 In contrast to previous work investigating the link between chemical contaminants and  
53 genetic diversity in variegated scallops (Breitwieser et al., 2016; Breitwieser et al., submitted-  
54 a), the present study is able, for the first time, to relate both inorganic and organic pollutants  
55 with genetic diversity measured in the same specimens across a large number of sites along  
56 the Atlantic coast. In addition, our study uses an innovative statistical analysis (Path-  
57 ComDim) to take into consideration the effect of chronic chemical pollution (pesticides,  
58 polycyclic aromatic hydrocarbons, polychlorobiphenyl and heavy metals) on (1) genetic  
59 diversity, and (2) the metabolic and physiological response through the combined use of  
60 several biomarkers of pollution (SOD, GST, MDA, Laccase) of *M. varia* specimens collected  
61 from 13 sites.

62 This study is an essential step toward monitoring the health of marine coastal environments  
63 along the French Atlantic coast. Our work follows the recent implementation of different  
64 strategies and operating procedures developed as part of cross-disciplinary policies on issues  
65 relating to the coast and the maritime economy, as it provides a measure of environmental  
66 quality through the use of a bioindicator marine bivalve.

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## 68 **2. Materials and Methods**

69

### 70 *2.1- Field collection and tissue sampling*

71 The study sites are shown in Fig. 1. Scallops were collected from 13 sampling sites from  
72 Brest down to the Nouvelle-Aquitaine Region. “Loix” was chosen as the less-contaminated  
73 site. The open areas, “Troveoc” and “Plougastel”, which are situated in Brest Bay, are  
74 subjected to anthropogenic pressure from urban activities and the Elorn estuary. “Erdeven”



75 and “Auray”, situated in southern Brittany, are subject to heavy contamination from  
76 agriculture and pig farming. “Port-Neuf” is situated near a water-treatment plant and the  
77 industrial area of La Rochelle. “Angoulins” may be exposed to currents and pollution from  
78 adjacent urban tourist areas. “Aix Isle”, “Les Palles” and “Oleron” are situated across the  
79 Charente estuary, which is impacted by sources of pesticide pollution, in particular from  
80 treatment of the vines.

81 Finally, for the semi-open areas, “outlet dredging” is an area of outlet dredging near a  
82 touristic beach and close to a harbour, “outlet rainwater” corresponds to the outlet of  
83 rainwater in a harbour (without a water-filtering system), “outlet fairing” is located below the  
84 careening area, which has a water-filtering system (carbon, sand, UV and paper to remove  
85 organic, inorganic and bacterial contamination).

86 Tissue samples from variegated scallops (*M. varia*) were collected from the 13 sites described  
87 above. For bioaccumulation and chemical biomarker assays, the digestive glands and gills  
88 were sampled from 130 individuals (10 individuals per site) collected in March 2016 (adult  
89 size mean of  $4.04 \pm 0.32$  cm). For the genetic approach, muscle tissue was sampled from the  
90 same 130 individuals and from additional variegated scallops collected at these sites in 2014  
91 and 2016, to estimate population diversity indices. Thus, genetic data were obtained from 459  
92 individuals (25 to 59 individuals per site) and these data are described in Breitwieser et al.,  
93 submitted-a).

94 Sampling and protein assays were carried out in March 2016. For each sampling site, the  
95 organs of the bivalves were removed *in situ* and stored at  $-80$  °C for further analysis. Each  
96 gill was cut into two samples and examined for trace elements and a biochemical assessment  
97 of biomarkers was performed; each digestive gland was cut into two samples in order to  
98 assess organic contaminants and perform a biochemical assessment of biomarkers  
99 (Breitwieser et al., 2016). Samples for trace element analysis (gills) were freeze-dried for 48

100 h. For each scallop, muscle tissue was collected and stored in absolute ethanol for genetic  
101 analysis.

102

### 103 2.2- *Assays of heavy metals*

104 Analyses of trace elements were based on previous work by Unrine et al. (2007). Assays  
105 of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, V and Zn were performed on gills with a  
106 Varian Vista-Pro ICP-OES and a Thermofisher Scientific XSeries 2 ICP-MS.

107

### 108 2.3- *Assays for organic contaminants*

109 The digestive glands of 7 scallops per site were analysed for 21 PAHs, 14 PCBs and 8  
110 pesticides by stir bar sorptive extraction-thermal desorption-gas chromatography-tandem  
111 mass spectrometry (SBSE-GC-MS/MS) using a method adapted from Lacroix et al. (2014).  
112 The results are expressed as µg analytes/kg dry weight (d.w.). Limits of quantification (LOQ)  
113 were calculated using the calibration curve method (Shrivastava et al., 2011) and the limit of  
114 detection (LOD) was estimated by dividing LOQ by 3. Analytical quality control was made  
115 using the Standard Reference Materials (1974c) “Organics in Mussel Tissue (*Mytilus edulis*)”  
116 provided by the National Institute of Standards and Technology (NIST, Gaithersburg, USA).

117

### 118 2.4- *Biochemical assays*

#### 119 2.4.1. *Sample processing*

120 For biomarker assessment in gills and digestive glands, samples were homogenized in  
121 ice-cold phosphate buffer (100 mM, pH 7.2, 1100 mOsm). The homogenates were centrifuged  
122 at 12,500g / 4 °C for 15 min and the Final Fractions (FF) were used for biochemical assays.

123

124            *2.4.2 Total protein concentration*

125            Total protein concentration was determined using an adaptation of the BCA Kit  
126 method (Bicinchononique Acid Kit, Sigma Aldrich). The kit contained bovine serum albumin  
127 (BSA) as a standard and involved the reduction of alkaline  $\text{Cu}^{2+}$  by proteins (Smith et al.,  
128 1985) at an absorbance of 562 nm using a spectrofluorometer (SAFAS Flx-Xenius).

129

130            *2.4.3 Antioxidant enzyme activity*

131            SOD activity was determined in the FF using the method developed by Paoletti et al.  
132 (1986). The results are presented in U of SOD/mg of protein.

133            GST activity in the organs was determined by adapting a Sigma assay kit method and  
134 the results are expressed in U of GST/mg of protein (Mannervik et al., 1988; Habig et al.,  
135 1974).

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137            *2.4.4 Laccase assay*

138            Using a method developed by Luna-Acosta et al. (2010), this activity was measured in  
139 the FF. The reaction was assessed at 420 nm using a spectrofluorometer (SAFAS Flx-  
140 Xenius). In parallel, the non-enzymatic auto-oxidation (oxidation in the absence of FF) was  
141 subtracted. Laccase-type PO activity is expressed as U of laccase/mg of protein (one unit is  
142 defined as the amount of enzyme that catalyses the appearance of 1  $\mu\text{mol}$  of product per min).

143

144            *2.4.5 Lipid peroxidation*

145            Damage, and in particular lipid peroxidation, was estimated by assessing the MDA  
146 concentration in the final fraction. The product was quantified by measuring absorbance at  
147 586 nm (Gerard-Monnier et al., 1998) using the SAFAS Flx-Xenius spectrofluorimeter. The  
148 results are expressed in  $\mu\text{M}.\text{mg protein}^{-1}$ .

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150       2.5- *Genetic approach*

151       Genetic diversity of scallops was assessed using sequences of a 444 base-pair portion of  
152 the mitochondrial gene coding for cytochrome c oxidase I (cox1). DNA extraction, PCR and  
153 sequencing protocols were described in Breitwieser et al. (2016) and Breitwieser al.  
154 (submitted-a). The haplotype diversity (Hd), and nucleotide diversity ( $\pi$ ) were calculated  
155 using Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010), for each sampling site  
156 separately.

157

158       2.6- *Data processing*

159       First, preliminary statistical analyses were carried out using R v. 3.1.2 (R Core Team,  
160 2016). In all cases, normality was tested on residuals using the Kolmogorov-Smirnov test and  
161 homogeneity of variance was assessed using the Bartlett test. Moreover, a multi-correlation  
162 analysis was performed to highlight correlations between chemical contaminant  
163 concentrations and biomarker values.

164       Second, a multiblock statistical analysis with a path modelling approach was used to take  
165 into account specific patterns of directed relations among the blocks of variables. In our case  
166 study, five blocks of variables were considered: assays for heavy metals, assays for organic  
167 contaminants, biochemical assays on the gills, biochemical assays on the digestive glands and  
168 genetic data. The advantage of the multiblock data method of analysis used herein, named  
169 ComDim (Mazerolles et al., 2002; Cariou et al., 2017), is to simultaneously exhibit common  
170 dimensions shared by the blocks and introduce a system of weighting that allows each block  
171 to have a specific weight for each common component. Moreover, as reported in Cariou  
172 (2017), the combination of this multiblock analysis with a path modelling approach, known as  
173 the Path-ComDim approach, takes into account the existence of an *a priori* specific pattern of

174 directed relations among the datasets at hand. Thus, Path-ComDim aims to reduce the  
175 dimensionality of data organized in several blocks and to estimate the importance of the  
176 assumed effects of one block on another block of information. For this analysis, we used  
177 contaminant and biomarker data obtained from 91 of the collected specimens, and the genetic  
178 diversity indices obtained for each site (estimated from 25 to 59 individuals per site) (Figure  
179 2).

180 We considered a path diagram with arrows directed from heavy metals to biochemical  
181 assays in the gills, digestive glands and the genetic data on the one hand, and organic  
182 contaminants to biochemical assays in both organs and genetic diversity, on the other (Figure  
183 2). These arrows reflect the fact that the chemical contamination measurements (heavy metals  
184 and organic contaminants) are assumed to have an influence on the health status measured by  
185 the biochemical assays and the genetic approach.

186 From a practical point of view, each block of data was subjected to a pre-treatment: at a  
187 given site, organic contaminants for which assay values were regularly under the LOQ  
188 (except for two or three observations) were summed. Thus, several PAH variables were  
189 aggregated into a variable named “PAHothersum”, and several PCBs were aggregated into  
190 the variable “PCBothersum”. All eight pesticides were also summed together. Afterwards all  
191 the variables were individually standardized and each block was set at the same total variance  
192 to account for the fact that the number of variables is different from one block to another.

### 193 **3. Results**

194 Data used in the Path-ComDim analysis are shown in the supplementary data (Tables  
195 S1 and S2, Figure S1). For this analysis, we used heavy metals contaminants (Figure S1),  
196 organic contaminants concentrations (Table S1), the genetic diversity indices obtained for  
197 each site (Table S2) and biomarker data (Table S3).

198 Table 1 illustrates the results of the first three common components, obtained using  
199 Path-ComDim. It turns out that the first two components (dim. 1 and 2) have an  
200 overwhelming importance (47.9 % for dim. 1 and 34.5 % for dim. 2) in comparison to the  
201 third component (7.0 % relative importance). Table 1 also shows the contribution of each pair  
202 of linked blocks of variables to the definition of each common component. It appears that the  
203 first common component (dim.1) mainly highlights the link between organic contaminants  
204 and the genetic data (99.3%). The second common component mainly highlights the link  
205 between heavy metals and biochemical assays in the digestive gland (83.8%), whereas a weak  
206 relationship was observed between organic contaminants and biochemical assays in this organ  
207 (8.6%). The links between heavy metals and biochemical assays in the gills, between heavy  
208 metals and genetic diversity and between biochemical assays and organic contaminants in the  
209 gills do not really contribute to the two first common components.  
210 The third component mainly accounts for the link between heavy metals and biochemical  
211 assays in the gills, but as the relative importance of this dimension is very small (0.4 %), this  
212 result will not be taken into account in the following analysis.

213 From now on, we shall focus on the first two components, denoted t1 and t2. Figure 3  
214 shows the configuration of the samples, in relation to their respective site, on the basis of t1  
215 and t2. Figure 3 also shows correlations between the common components t1 and t2 and the  
216 variables according to the block they belong to (heavy metals, organic contaminants,  
217 biochemical assays in the gills and digestive gland, genetic approach). As each common  
218 component is built to take account as much as possible of the relationship between the blocks  
219 of variables, the subplots 3b to 3f in Figure 3 make it possible to illustrate the links between  
220 the blocks of variables depicted in Table 1.

221 Concerning the block of heavy metal variables (Figure 3b), three clusters of heavy  
222 metals can be observed along the second component, t2, namely Cu/Se/Zn/As, Cd/Ag, and

223 Pb/Cr/Co/Fe/Mn/Ni. This partition was confirmed using a clustering approach for variables  
224 (Vigneau et al., 2015). The Sn and V values were not taken into account because of the lower  
225 quantification with respect to the LOD value. The three clusters of heavy metals seem to be  
226 associated with location. Indeed, the sites “Outlet rainwater” and “Outlet Fairing”, which are  
227 situated in a harbour area, could modulate the Cu/Se/Zn/As cluster, whereas “Outlet  
228 dredging” and the majority of the sites situated near the Charente Estuary (“Oleron”,  
229 “Angoulins”, “Port-Neuf”, “les Palles”) could influence the Pb/Cr/Co/Fe/Mn/Ni cluster.

230 Moreover, it was interesting to show (Table 1) the strong contribution link between  
231 heavy metals and biochemical assays in the digestive glands. Indeed, the Path-ComDim  
232 model showed that the biochemical values for the digestive gland had a common dimension  
233 (were of a similar order of magnitude) on axe 2 (Figure 3e). In the digestive glands, there was  
234 a positive correlation between the biomarkers GST and MDA, but a negative correlation  
235 between the group GST/MDA and the Laccase biomarker. The SOD biomarker involved in  
236 oxidative stress did not correlate with the other pollution biomarkers (laccase, MDA, GST).

237 The organic contaminants (pesticides, hydrocarbons, polychlorobiphenyls) are well  
238 depicted along the common dimension t1. The correlation plot on Figure 3c shows that the  
239 Polycyclic Aromatic Hydrocarbon (PAH) variables (except biphenyl), several PCB variables  
240 (PCB28, PCB77 and the sum of the rare PCBs) and the variable summing all the pesticide  
241 assays correlated well (negatively) with t1. All organic contaminants are situated to the left-  
242 hand side of the plot, and in greater quantity in the “Plougastel” site (for pesticides and a rare  
243 PCB group named “PCB other sum” in Figure 3c), “Outlet fairing”, “Outlet rainwaters” and  
244 “Outlet dredging” sites concerning PCB 28, PCB 77 and all hydrocarbons (Figures 3.a and  
245 3.c).

246

247 The two genetic variables on Figure 3f (haplotype diversity and nucleotide diversity)  
248 are positively correlated with t1 and are expressed more in sites such as “Loix”, “Angoulins”,  
249 “Les Palles” and “Oleron” on the right-hand diagram (Figure 3a). The genetic variables are  
250 not correlated with t2 and thus seem not to be linked with heavy metal contaminants (Figure  
251 3b). In contrast, t1 illustrated the negative relationship between organic contaminants (Figure  
252 3c) and the genetic block (Figure 3f). The greater the quantity of organic contaminants, the  
253 lower the indices of genetic diversity (haplotype diversity, nucleotide diversity). This link  
254 contributed greatly to t1, as already shown in Table 1.

255

256 It is interesting to note that the results of the biochemical assays on the digestive  
257 glands (Figure 3e) were different to those in the gills (Figure 3d). Indeed, it seems that there  
258 was a significant background noise in the results of the biochemical assays on the gills. As a  
259 consequence, the block of biochemical assays on the gills appeared to have no strong links  
260 with the other blocks.

261 These analyses are particularly interesting in comparison with heavy metals, which had a link  
262 contribution of 83.8% on common dimension 2 (Table 1). Indeed, a greater contamination of  
263 Co, Cr, Pb and Ni led to a positive immunomodulation in the “Outlet dredging” site, whereas  
264 a decrease in the specific activity of laccase was influenced by As and Se at “Plougastel”.  
265 Moreover, the higher contamination with As and Se induced a higher MDA and GST  
266 response, both of which are involved in lipid peroxidation and the detoxification of xenobiotic  
267 compounds, unlike laccase.

268

#### 269 4. Discussion

270 In this work, an innovative analysis of the effects of chemical contamination (inorganic  
271 and organic pollutants) on the biology of the variegated scallop *M. varia* was carried out using



272 a statistical approach involving the Path-ComDim model to: i) clearly study responses to both  
273 contaminants (heavy metals and organic pollutants); ii) identify the biochemical and genetic  
274 effects for each type of contaminant; and iii) understand the combined effect of the  
275 contaminants on health status.

276

#### 277 4.1- Chemical contaminants

278 Our study was carried out using scallops collected *in situ* in March 2016, and for each  
279 specimen, the level of contamination with heavy metals and organic pollutants was estimated  
280 in the gills and digestive glands. A small number of bio-monitoring investigations of coastal  
281 waters have been carried out by the local stakeholders on the Atlantic coast to evaluate the  
282 environment quality (e.g. the water department of the local authorities, the harbour  
283 authorities). Using the variegated scallop *M. varia*, our results showed higher concentrations  
284 of Pb, Cr, Fe, Ni, Co in the gills of this bivalve in the Pertuis Charentais (“Oleron”,  
285 “Angoulins”, “Port-Neuf”, “Les Palles” sites) and in particular in “Outlet dredging” zones in  
286 La Rochelle harbour in comparison with the least-contaminated area, “Loix”, described as a  
287 reference site in the literature (Bustamante and Miramand, 2005). Moreover, Cu and Zn  
288 concentrations were higher in “Troveoc”, “Erdeven”, “Auray” and “Outlet rainwater” than in  
289 “Loix”. These results confirm the hypothesis that the Atlantic coast is contaminated by trace  
290 elements from anthropogenic activities in wastewaters and inputs from drainage areas directly  
291 adjacent to the sea, and watershed-derived inputs (e.g erosion of contaminated land, effluents  
292 from highly-urbanized rivers, domestic and/or industrial effluents from wastewater treatment  
293 plants, urban runoff and sewers). Furthermore, the capacity of heavy metal contaminants to  
294 bio-accumulate in bivalve molluscs (Bryan, 1973) depends on the main function of each  
295 organ i.e. trapping nutrients (gills), or digesting and excreting nutrients (digestive gland).  
296 Indeed, previous studies have shown a greater bioaccumulation of chemical contaminants in

297 the digestive gland in comparison with the gills (Breitwieser et al., submitted-b). However, it  
298 is important to underline the difference between non-essential and essential metals: non-  
299 essential heavy metals such as As, Pb, Co, Cd could be more toxic for the scallop (Rainbow et  
300 al., 2002) and on a larger scale, for Human. In fact, these trace elements are used particularly  
301 in industrial sectors, wood preservatives, electrical battery manufacturing, alloy production,  
302 and are classified in the first group of carcinogenic compounds (International Agency for  
303 Research on Cancer). According to a report by the National Institute for Public Health (Tome  
304 2: heavy metals and metalloids, December 2017), their major impregnation by human could  
305 be due to seafood consumption.

306         Organic contaminant levels in the digestive gland of *M. varia* scallop provided  
307 information on anthropogenic sewage in 13 separate sites on the Atlantic coast. In this work,  
308 the data obtained showed high levels of pesticides in Plougastel (sum of dieldrin, aldrin,  
309 isodrin, endrin, 2-4 dde, 4-4 dde and rare PCBs (52, 101, 135, 118, 153, 105, 138, 156, 180,  
310 169)) collected together in a single variable (Figure 3c). This might be explained by the slow  
311 and insidious degradation of these components, and in particular PCBs, which are now  
312 prohibited because they are responsible for acute toxicity. Concerning PCBs 28 and 77,  
313 measurements in the digestive glands of *M. varia* indicated that the mean of these two PCBs  
314 was significantly higher in Plougastel, outlet rainwater and fairing areas. Moreover,  
315 measurements of pyrene and other hydrocarbon congeners in the digestive glands showed  
316 anthropogenic pressure (S1 Table of supplementary data). These results might be due to the  
317 proximity of urban zones along the Atlantic coast, despite the fact that all of these  
318 contaminants are still considered as a priority by the WFD in France.

319         Indeed, on the correlation plot on Figure 3c, all the contaminants are situated on the  
320 left of the plot in the common dimension 1. All the organic components could potentially  
321 originate from various activities in these sectors (fishing harbours, tourist harbours, petroleum

322 refinery, waste treatment plant) with tidal currents, particularly from the Gironde Estuary and  
323 from Brittany (Luna-Acosta et al., 2017). Moreover, previous research indicated that the  
324 concentration of pesticides, PCBs and PAHs in sediments was higher than in the surrounding  
325 water, and this might account for the observation in the digestive glands of *M. varia*, which is  
326 a sedentary benthic species (Luna-Acosta et al., 2017).

327         These data are in agreement with previous results showing that other marine bivalves  
328 (mussels, oysters and scallops) collected on the Atlantic coast have a strong ability to ingest  
329 and bio-accumulate more hydrophobic high molecular weight PAHs and heavy metals in their  
330 digestive organs than in other organs as gills and mantle (Luna-Acosta et al., 2017;  
331 Breitwieser et al., 2017, Lacroix et al., 2017).

332

#### 333         4.2- *Health status response*

334         Because benthic marine molluscs are exposed to multiple stressors such as contaminant  
335 cocktails and it is not always possible to test for their presence within tissues, there has been a  
336 search for indicators that demonstrate the toxicity of chemical pollutants. The advantage and  
337 originality of our study is that indicators of health status and contaminants were assessed  
338 using the Path-ComDim statistical model in distinct tissues. As previously suggested in *M.*  
339 *varia*, indicators of health status are pollutant biomarkers for the short-term (GST, MDA,  
340 SOD, Laccase). It is for this reason too that biomarkers are currently being used to assess the  
341 condition of European coastal waters (Hagger et al., 2009).

342         From the results obtained with the Path-ComDim model, the biomarkers in the gills of the  
343 variegated scallop were not modulated, in contrast to the biomarkers in the digestive gland  
344 and the genetic diversity indices. This may be linked to the fact that the ability to deal with  
345 oxidative stress, detoxification and immunomodulation revealed by pollution biomarkers may  
346 depend on the accumulation and the kind of contamination (Hannam et al., 2010).

347 In this work, the biomarkers of oxidative stress used were SOD and MDA. It was  
348 demonstrated that these indicators could provide some of the first signals of stress in molluscs  
349 (Regoli et al., 2000; Valavanidis et al., 2006). Furthermore, GST is a phase II conjugation  
350 enzyme that intervenes in the metabolism of xenobiotics and in reactions which are catalysed  
351 by membranes, or cytosolic enzymes. According to previous studies, GST-specific activity  
352 increases in exposed organisms in relation to the concentration of xenobiotics in the medium.  
353 Indeed, GSTs, which correlated positively with MDA in this study, are enzymes whose  
354 activity is used as a biomarker of exposure to organic substances, especially in molluscs,  
355 where EROD activity (a marker of cytochrome P450 involved in the metabolism of  
356 potentially toxic compounds) is not routinely assessed (Cajaraville et al., 2000). Laccase  
357 proved to be a sensitive biomarker and phenoloxidases seem to play an important role in  
358 bivalve immunity when they are activated by ROS (Luna-Acosta et al., 2010). Moreover,  
359 Laccase contains metal coenzymes which can be affected by inorganic contamination (heavy  
360 metals) in a similar fashion to the activation pathway of SOD in the short term, which  
361 explains the contrasting response of this enzyme activity to that of the other biomarkers, GST  
362 and MDA.

363 We proposed to use genetic diversity as an indicator of population health because  
364 populations with high levels of genetic variation tend to have a better viability (Frankham et  
365 al., 2002), a better capacity to resist environmental stress (e.g. Nowak et al., 2012) and  
366 possess a greater adaptive potential when facing environmental changes (Eizaguirre and  
367 Baltazar-Soares, 2014). Since historical processes and dispersal (i.e. gene flow) influence the  
368 genetic diversity of populations, information on the species phylogeography and population  
369 structure is required to interpret differences in genetic diversity across sampling sites  
370 (Theodorakis, 2003). With that goal, we recently assessed the genetic structure of *M. varia*  
371 using *cox1* sequences and did not detect genetic differentiation among sites along the French

372 coasts of the Bay of Biscay and English Channel (Breitwieser et al., submitted-a). Results  
373 based on this mitochondrial DNA marker suggest that all individuals sampled in the present  
374 study belong to the same population and that differences in genetic diversity observed among  
375 our sampling sites (Supplementary Table S2) are not due to genetic drift or historical events.

376 Different regions of France and many countries of the European Union have established  
377 an integrated ecosystem-based approach to the management of marine resources. Any  
378 assessment of the cumulative short-term or long-term effect of all the activities that can  
379 potentially impact an environment that is prone to large natural variations is a relevant but  
380 difficult task. In recent years, pragmatic approaches have been initiated by urban authorities,  
381 and local and regional stakeholders of Charente-Maritime (France). The aim of these  
382 voluntary actions is to monitor quantitative chemical concentrations but not to study the  
383 qualitative effects on environmental quality. Today, several pollutant biomarkers are being  
384 employed in biomonitoring studies but the results are difficult to compare. This is why our  
385 research was focused on refining and standardising current biomarker techniques for specific  
386 contaminant types using a statistical model as this will allow comparisons to be made.

387

#### 388 *4.3- Relationship between health status and chemical contaminants*

389 Thanks to the Path-ComDim model, a comprehensive analysis of our results could be  
390 performed. Based on previous research, the discussion will be divided into two parts to  
391 explain the relationship between health status and contaminants in this study. In contrast to  
392 others studies (Breitwieser et al., 2016), all heavy metals and organic pollutants were  
393 considered in the model because all of the data come from the same 91 specimens, which is a  
394 relevant approach.

395 Concerning biomarkers of pollutants, only the results in the digestive gland showed a  
396 significant response with heavy metal contaminants. Our model accounts for 83.8 % of link

397 contribution, suggesting that MDA, which reflects lipid peroxidation, and GST, which shows  
398 detoxification of xenobiotics, were positively correlated and were modulated by Cu, Se, As  
399 and Zn. Furthermore, based on the model, these four heavy metals, and a group of specific  
400 organic contaminants (pesticides, and PCBs 28 and 77), could significantly diminish Laccase  
401 activity, in agreement with previous work (Luna-Acosta et al., 2017; Luna-Acosta et al.,  
402 2015; Milinkovitch et al., 2015). These results could explain the high functionality of the  
403 xenobiotic bio-transformation process by GST on the one hand, and also the decrease in anti-  
404 oxidant protection and immune modulation by an increase in MDA and a decrease in Laccase  
405 on the other hand (Pan et al., 2005; Pan et al., 2009; Breitwieser et al., 2016). These enzyme-  
406 specific activities and concentration levels could be related to the high contaminant  
407 concentrations in the harbours, outlet rainwater and touristic areas (Breitwieser et al., 2017).  
408 However, it should not be forgotten that abiotic factors could modulate biomarker activities  
409 and bioaccumulation. Indeed, data on physico-chemical parameters collected at the same date  
410 are shown on a map of work presented in Breitwieser et al., (submitted-a). Only a significant  
411 difference in salinity was discernible between Erdeven, Auray and all other sites, but these  
412 two sites are mixed with others on the plot of sites (Figure 3a).

413 Concerning genetic effects of pollution, it has already been stressed that chemical  
414 contaminants can reduce genetic diversity (e.g. Nowak et al., 2009), or induce deleterious  
415 mutations that can be heritable (e.g. Cronin and Bickham, 1998), and per consequence, can  
416 impact the population health (Bickham et al., 2000; Bickham, 2011). However, some studies  
417 have observed greater genetic diversity in polluted environments (Pedrosa et al., 2017)  
418 illustrating that the relationship between chemical contamination and genetic diversity is not  
419 straightforward and will depend on the species demography, dispersal, and level of  
420 contamination.

421 In our study, low levels of genetic diversity were clearly correlated with high levels of  
422 organic contaminants in variegated scallops from outlets fairing, dredging and Plougastel.  
423 Since gene flow does not seem to be restricted among our sampling sites (Breitwieser et al.,  
424 submitted-a), a reduction in genetic diversity at more polluted sites cannot be explained by a  
425 population bottleneck. Conversely, selective mortality of individuals possessing  
426 mitochondrial haplotypes susceptible to organic pollutants is plausible. Indeed, mitochondria  
427 seem to be particularly vulnerable to environmental contaminants (Meyer et al., 2013 and  
428 references therein), therefore natural selection may be acting on the mitochondrial genome in  
429 organisms exposed to chemical contaminants.

430 To further test our hypothesis, extensions of this study could include i) obtaining genetic  
431 diversity estimates from neutral markers (nuclear loci not under natural selection) and  
432 conducting a more fine-scale investigation of gene flow among sites using multiple  
433 independent genetic markers such as microsatellites, and ii) assessing experimentally  
434 differential mortality of distinct mitochondrial haplotypes following organic contaminant  
435 exposure. Additionally, genome-wide data could be used to investigate local adaptation to  
436 pollution (Oziolor et al., 2017), which may occur despite gene flow (Tigano and Friesen,  
437 2016). Finally, our study focused on the adult stage *in situ*, but chemical contamination also  
438 affects early life stages (larvae and juveniles) in bivalves (e.g. Calabrese et al., 1977). Thus, a  
439 multigenerational controlled experiment submitting variegated scallops to chemical  
440 contaminants, and measuring mortality and genetic diversity of the different life-stages would  
441 provide further insights on the resistance and potential adaptation of this bioindicator species  
442 to contamination.

443 Overall, the statistical model used for monitoring health status is relevant. Indeed,  
444 contrary to other models such as PLS-Path Modelling (Tenenhaus et al., 2005; Bertrand et al.,  
445 2017) which extract one dimension by block and fit a unique common model, the Path-

446 ComDim model produces several common dimensions, each of them defining specific  
447 weights for the blocks with regard to the links investigated. Furthermore, the graphical output  
448 of Path-ComDim can be read like a principal component analysis (PCA). This leads to rather  
449 simple and interpretable media for the scientific community.

450

## 451 **5. Conclusion**

452 In conclusion, the maritime economy serves a wide range of diversified activities, both  
453 traditional (fishing, shell fishing, harbour activities, tourism etc.) and emergent (renewable  
454 marine energy, biotechnology etc.). In addition to these activities, which are the mainstay of  
455 the maritime economy, indirect activities such as logistics, industrial port zones and transport  
456 have to be considered. In France, and in particular in the majority of our study sites, the south  
457 Atlantic seaboard, with its 720 kilometres of coastland and the Charente-Maritime islands, is  
458 an attractive space. Out of interest, the population is constantly growing in the French South-  
459 Atlantic, at a rate of 9.5% between 1999 and 2011, in comparison with the population growth  
460 in France, which was 7.9% during the same interval.

461 In recent years, shellfish farmers have experienced repeated crises, and this has raised  
462 serious issues. France is the biggest oyster producer and the third biggest mussel producer in  
463 Europe according to the “Comité National de la Conchyliculture, [http://www.cnc-](http://www.cnc-france.com)  
464 [france.com](http://www.cnc-france.com)”. Apart from the health risk, a decrease in the quality of the water, the living  
465 environment of marine species, would damage marine environments, biodiversity and the  
466 development of economic stakeholders (the aquaculture sector, fishing and also tourism). So,  
467 it is now crucial to include an assessment of coastal water quality no less frequently than  
468 annually. Indeed, using new tools such as Path-ComDim applicated in this study, it is now  
469 necessary to address coastal water contamination; this has been undertaken by some harbour



470 authorities, in particular in La Rochelle, which created a Department of the Environment 10  
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472

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483

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**Table 1:** Importance of the three first common components of Path-ComDim and the contribution of each pair of kinked blocks to the common components.

Importance of the common components		Contribution of each link (%)							
dimension	Absolute importance	Relative importance for each dim.	→	Heavy metals to BCH gills	Heavy metals to BCH dg	Heavy metals to genetic approach	ORG to BCH gills	ORG to BCH dg	ORG to genetic approach
1	0.0080	47.9%	→	<0.1%	<0.1%	0.5%	<0.1%	0.1%	<b>99.3%</b>
2	0.0057	34.5%	→	0.3%	<b>83.8%</b>	5.2%	<0.1%	8.6%	2.10%
3	0.0012	7.0%	→	<b>96.8%</b>	2.6%	<0.1%	0.5%	<0.1%	<0.1%

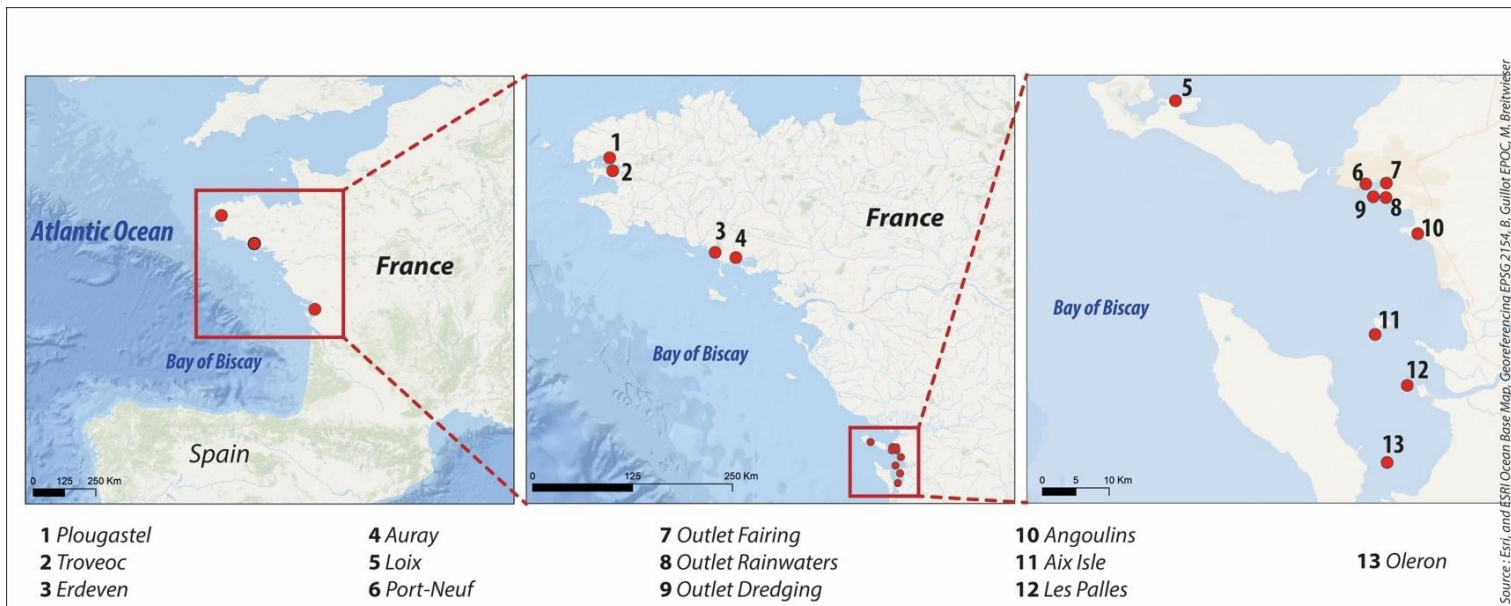
**Caption for table 1:**

*BCHdg: biochemical assays in the digestive glands*

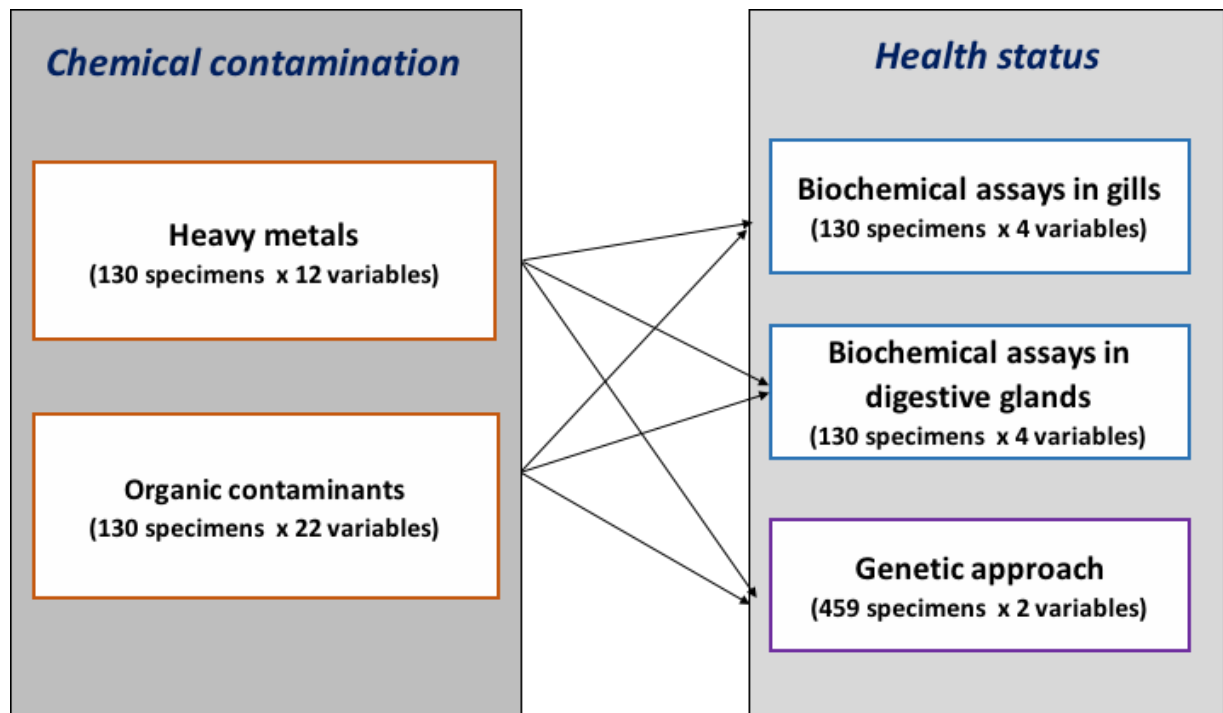
*BCHgills: biochemical assays in the gills*

*ORG corresponds to the organic contaminants*

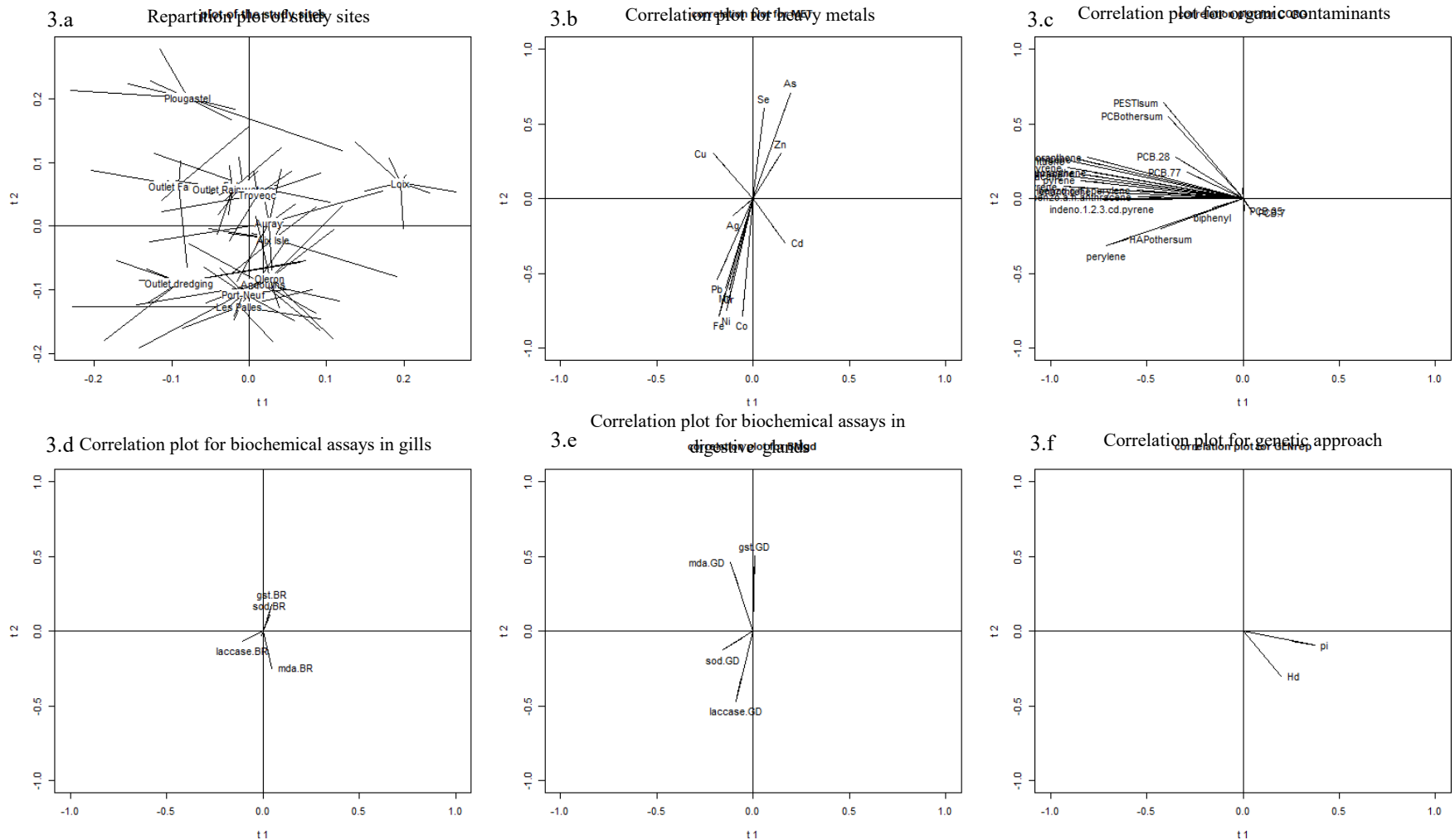
*Values in the first part of table (importance of the common components) were evaluated as the sum of squares of the saliences of the blocks to the common component extracted at each step (i.e. dimension)*



**Figure 1:** Map of France with the location of the study sites in the Brittany and Nouvelle-Aquitaine regions.



**Figure 2:** Path diagram for the five data blocks report summarizing chemical contamination with two blocks (heavy metals and organic contamination) and health status with three blocks (biochemical assay in two organs and the genetic approach). For each block, we analysed the same 130 specimens for bioaccumulation and biochemical aspects and 459 individuals (25 to 59 per site) for genetic study of the variegated scallop *Mimachlamys varia*.



**Figure 3:** Multiblock dataset (Path-ComDim) analysis of biomarker responses (MDA: MDA contents, SOD: SOD activity, GST: GST activity, LAC: Laccase activity) and genetics, heavy metals, and organic contaminants accounting for the variation in the individual biomarker matrix for individuals of *M. varia* collected in March 2016 at 13 sampling sites.

