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1 **Differences in chemical contaminants bioaccumulation and ecotoxicology biomarkers in *Mytilus***  
2 ***edulis* and *Mytilus galloprovincialis* and their hybrids**

3

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15

16 **Highlights**

17 - Bioaccumulation and biomarker responses differ between *Mytilus* species in the field

18 - Variations in contaminant levels were observed between mussel species

19 - Differences were observed between mussel species in biomarkers response

20 - Genetics data are an important additional parameter for biomonitoring programs

21

22 **Abstract**

23 The *Mytilus* mussels are spread all over the world and many related species coexist in several areas  
24 and can produce hybrid offspring. Mussels have been used for decades in national and  
25 international programs to monitor chemical contamination in the environment. Differences in  
26 bioaccumulation and biotransformation abilities between species and their hybrids should be  
27 evaluated to assess the comparability of the results obtained within the international  
28 biomonitoring programs. The objective of this study was to characterize bioaccumulation abilities  
29 and biomarker responses in *Mytilus edulis*, *Mytilus galloprovincialis* and their hybrids via an *in situ*  
30 transplantation experimentation on their progenies. Four mussel groups (*M. edulis*, *M.*  
31 *galloprovincialis* and two hybrids batches) issued from genetically characterized parents were  
32 transplanted for one year in Charente Maritime (France) to ensure their exposure to identical  
33 sources of contamination. The bioaccumulation of several families of contaminants (trace metals,  
34 polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, polychlorinated biphenyls), the  
35 response of several biomarkers (DNA strand breaks level, lysosomal membrane stability,  
36 metallothionein content, acetylcholine esterase activity) and some physiological parameters

37 (growth, mortality, gonadal development), were analyzed. Differences were observed between  
38 species, however they were contaminant-specific. Variations in contaminants levels were observed  
39 between progenies, with higher levels of Cu, PBDE, PCB in *M. edulis*, and higher levels of Cd, Hg, Zn  
40 in *M galloprovincialis*. This study demonstrated that variations in contaminant bioaccumulation and  
41 different biomarker responses exist between *Mytilus* species in the field. Data on species or the  
42 presence of hybrid individuals (or introgression) is an important additional parameter to add to  
43 biomonitoring programs databases.

44

45 **Keywords**

46 mussel, hybridization, genetics, metal trace elements, biomonitoring

## 47 **1. Introduction**

48 The genus *Mytilus* is spread all over the oceans. In the Northern Hemisphere, three closely related  
49 species have been identified: *Mytilus edulis*, *Mytilus galloprovincialis* and *Mytilus trossulus*. About  
50 3.5 million years ago, *M. trossulus* and *M. edulis* diverged genetically, then around 2.5 million years  
51 ago, *M. edulis* and *M. galloprovincialis* diverged (Fraïsse et al., 2014). Since then, species have  
52 colonized new geographic areas and are now described in many parts of the world (Springer and  
53 Crespi, 2007). Although the species had initially distinct areas of distribution, the existence of  
54 sympatric zones and hybridization phenomena (the offspring resulting from combining two  
55 organisms of different specie) between species has been recorded (Bierne et al., 2003). Different  
56 genetic markers have been proposed to differentiate the three species (Inoue et al., 1995; Kijewski  
57 et al., 2011; McDonald et al., 1991), but the existence of introgression phenomena (the movement  
58 of genes of one species into the genetic pool of another by hybridization resulting in a complex  
59 mixture of parental genes), makes the species characterization difficult and only the use of several  
60 markers offers a reliable diagnosis (Bierne et al., 2003; Kijewski et al., 2011). In particular, the  
61 French Atlantic coast is characterized by the existence of complex "mosaic hybrid zones" in which  
62 *M. edulis* and *M. galloprovincialis* coexist (Bierne et al., 2003), as well as their hybrids and  
63 introgressed individuals.

64 Mussels have been used for decades in national and international marine biomonitoring programs  
65 (Kimbrough et al., 2008; Marigómez et al., 2013; ROCCH, 2016) as they are sessile filter-feeding  
66 animals accumulating contaminants in their surrounding environment (Beyer et al., 2017;  
67 Farrington et al., 2016). It has been frequently assumed that the mussels collected from the field  
68 were distinct species depending on the sampling site without considering potential interspecies  
69 differences in bioaccumulation. Physico-chemical parameters (i.e. salinity, tidal height, wave  
70 exposure) drive the species distribution (Bierne et al., 2002; Gardner et al., 1993), and as well as  
71 their external morphology (Akester and Martel, 2000; Seed, 1968). The mosaic distribution of  
72 *Mytilus* species, hybrids and introgressed animals challenges this initial spatial/morphological  
73 segregation. So far, only few studies addressed the issues of interspecies differences regarding  
74 bioaccumulation or biomarker responses. Higher concentrations of trace metals were found in *M.*  
75 *trossulus* compared to *M. edulis in situ*, but bias due to age of the sampled mussels were  
76 pinpointed (Lobel et al., 1990). In a laboratory study differences between *Mytilus* sp. in terms of  
77 copper bioaccumulation were demonstrated (Brooks et al., 2015), *M. edulis* and *M. trossulus*  
78 accumulating three times more copper than *M. galloprovincialis* over a 21-day exposure to 10 µg.L<sup>-1</sup>.  
79 However, the duration of the experimentation was short, under laboratory-controlled conditions  
80 and only copper at high concentrations was studied. This study also highlighted differences in  
81 ecotoxicological biomarkers, such as micronuclei, lysosomal structural changes and intracellular

82 accumulation of neutral lipids. Other studies pinpointed several physiological differences between  
83 mussel species such as spawning (Secor et al., 2001), growth (Fuentes et al., 2002; Lobel et al.,  
84 1990), parasite infestation (Secor et al., 2001), responses to salinity and temperature (Lockwood et  
85 al., 2010; Lockwood and Somero, 2011).

86 The aim of the present study was to assess if bioaccumulation differences and biomarker responses  
87 exist between *Mytilus edulis*, *M. galloprovincialis* and their hybrids or introgressed populations, via  
88 a one-year *in situ* transplantation experimentation using hatchery-produced mussels whose genetic  
89 background is known. The originality of this study consists in comparing genetically characterized  
90 mussel species and hybrids responses in the field, allowing to grasp the genetic response. This  
91 experimental design also avoid confounding factors such as age (e.g. the older mussel could  
92 accumulate higher contaminant concentrations, here all individuals have the same age) and  
93 differential acclimation to laboratory or field conditions (here parents having different  
94 origins/genetics were maintained in the same conditions since 2016 and all tested individuals were  
95 reared in the same location).

96 The bioaccumulation of several families of contaminants (trace metals, polycyclic aromatic  
97 hydrocarbons, PAH; polybrominated diphenyl ethers, PBDE; polychlorinated biphenyls, PCB) were  
98 analyzed in mussels. In addition, several ecotoxicology biomarkers (DNA strand breaks and  
99 micronuclei levels for genotoxicity; acetylcholinesterase activity for neurotoxicity; lysosomal  
100 membrane stability for general stress response; metallothioneins content for metal  
101 bioaccumulation capability) were analyzed. These bioaccumulable contaminants and  
102 ecotoxicological biomarkers are recommended by CEMP/OSPAR (2018) and ICES (Davies and  
103 Vethaak, 2012), and they are also identified as priority substances under the European Water  
104 Framework Directive. These biomarkers are monitored in France in fish and mussels as part of the  
105 European Marine Strategy Framework Directive. In addition, supporting parameters such as carbon  
106 and nitrogen stable isotopes and physiological parameters (growth, mortality, gonadal maturation)  
107 were measured in order to have a global view on the analyzed individuals.

108

## 109 **2. Material and Methods**

### 110 **2.1 Biological material**

111 Four groups of mussels were produced from wild populations sampled in four sites along the  
112 French coasts, each with a different proportion of *M. edulis* and *M. galloprovincialis* genotypes.  
113 These groups were part of the Ifremer MORBLEU project (Pepin et al., 2019). Briefly, wild mussels  
114 were collected between October and December 2016 along the French Atlantic coast (Biarritz, BIA,  
115 43.467448 N, -1.576547E; Ile d'Yeu, YEU, 46.70645 N, -2.284001 E; Moguéric, MOG, 48.688292 N,  
116 -4.070776E; Wimereux, WIM, 50.786667 N, 1.601944 W, see Appendix A 1 for details), then

117 transported to the Ifremer facilities in La Tremblade and kept in common controlled conditions  
118 suitable for germ cell maturation. All parents were genotyped to determine the species (and level  
119 of introgression) to which they belonged as described by Simon et al. (2019). Genotyping was  
120 performed with the KASP™ method (Semagn et al., 2014) on 77 nuclear markers in order to  
121 differentiate *M. edulis*, *M. galloprovincialis*, hybrids and introgressed individuals. Genetic clustering  
122 to determine admixture to either species was performed with the STRUCTURE software (Pritchard  
123 et al., 2000), these results are part of those published by Simon et al. (2019). The genetic structure  
124 analyses for the studied populations indicated that individuals from Wimereux (WIM, North France)  
125 were mainly *M. edulis* (the assignment to *M. edulis* is 99% for females and 88% for males);  
126 individuals from Moguéric (MOG, Northern Brittany) had a genetic profile of mainly Atlantic *M.*  
127 *galloprovincialis* introgressed with *M. edulis* (the assignment to *M. edulis* is 19% for females and  
128 11% for males) ; individuals from the Ile d'Yeu (YEU, West coast) presented introgressed *M.*  
129 *galloprovincialis* individuals, *M. edulis* individuals and first generation hybrids the (assignment to *M.*  
130 *edulis* is 52% for females and 52% for males); individuals from Biarritz (BIA, Basque coast) were  
131 mainly Atlantic *M. galloprovincialis* (assignment to *M. edulis* is of 0.01% for females and 8% for  
132 males) (see Appendix A for details).

133 In January and February 2017, Ifremer facilities in La Tremblade, parents were individually placed in  
134 a 300-mL beaker filled alternatively with unheated (10°C) or heated seawater to 20°C to induce  
135 spawning. Then, 2 males were mated with 1 females within population (9 males x 18 females for  
136 WIM, 6 males x 12 females for MOG, 10 males x 20 females for YEU and BIA), and embryos were  
137 transferred to 30-L tanks. Progenies of each group (WIM, MOG, YEU, and BIA) were reared in  
138 Ifremer Bouin hatchery and nursery until October 2017 when they reached  $19 \pm 2$  mm. Then,  
139 sampling for initial point (T0, 9 to 10-month old) was performed in November 2017, and mussels  
140 were transferred to the field in the Marennes-Oléron Bay (45.803251 N, -1.155008E) in baskets  
141 (one *per* group). Sampling occurred in March after 5 months (T5, 14-15-month old) and November  
142 2018 after 12 months in the field (T12, 22-23-month old). According to the last published Ifremer  
143 report on the Marenne Oléron bay (Morin et al., 2018), the site used in this study (called 'Mus de  
144 Loup' in the biomonitoring program) has levels of contaminants around or above the national  
145 medians for almost all studied compounds (except silver).

## 146 **2.2 Genetic markers**

147 In order to confirm the genetic background of the four groups, PCR on the genomic DNA for the  
148 GLU-5' (Inoue et al., 1995), SNP-001 and SNP-064 markers (Simon et al., 2019) were performed on  
149 the sampled individuals in March 2018 (T5, 10 individuals per group) and in November 2018 (T12,  
150 15 individuals per group, the same individuals were used for the biomarker responses, except for  
151 MTs). After the extraction of the genomic DNA from the frozen tissues (EZNA® Mollusc DNA Kit,

152 OMEGA bio-tek), the different PCR were carried out under the following conditions: 200 nM  
153 primers (see Appendix B), 200  $\mu$ M dNTPs, 1.5 mM MgCl<sub>2</sub>, 1X enzyme buffer (6.70 mM Tris-HCl pH  
154 8.8, 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01% Tween 20, 2.5 U Taq polymerase (Ozyme)), 200 ng d gDNA. The  
155 following programs were used on a Labcycler<sup>®</sup> thermocycler from Sensoquest: for GLU-5': 95°C-2  
156 min, [94°C-30s, 56°C-30s, 72°C-60s] x 40 cycles 72°C-5 min; for SNP-001 and SNP-064: 95°C-2 min,  
157 [94°C-30s, 60°C-30s, 72°C-30s] x 40 cycles, 72°C-2 min. After migration on agarose gel at 2% of the  
158 PCR products, it was possible to discriminate the alleles of the GLU-5' marker for the species *M.*  
159 *edulis* and *M. galloprovincialis* thanks to the size of the amplification products obtained (Inoue et  
160 al., 1995). For SNPs, since specific primers to each species were used, the alleles of both species  
161 were discriminated by the presence/absence of the amplification product on the gel. Results are  
162 expressed as a percentage of individuals with either *edulis* or *galloprovincialis* allele (homozygotes)  
163 or both (heterozygous individuals) for each marker.

### 164 **2.3 Physiological parameters**

165 At T5, shell length was measured and at T12, total weight, flesh weight and shell weight were  
166 recorded on 30 mussels per group in order to calculate the Meat yield [(fresh meat weight/total  
167 weight)\*100]. Mortality of each group was evaluated in February, April, and June 2018. At T12, 10  
168 mussels per group were analyzed by histological techniques to determine the gametogenesis stage  
169 in order to highlight any differences in the reproductive cycle. Flesh was removed from the shell  
170 and individually fixed in Davidson's solution (10% glycerol, 20% formaldehyde, 30% ethanol at 95%,  
171 30% sterile seawater, 10% acetic acid) for 48 h, then in 70% ethanol until sample processing.  
172 Samples were then dehydrated in a Leica TP 1020 automaton by successive baths of ethanol,  
173 butanol and paraffin. Several 3- $\mu$ m sections were individually deposited on a microscope slide and  
174 stained according to the Prenant-Gabe trichrome or Feulgen staining protocols (Gabe, 1968)  
175 allowing individual determination of sex and stage of gametogenesis based on the criteria  
176 previously described by Berthelin et al. (2000): quiescent stage (stage 0), start of gonial mitosis  
177 (stage I), meiosis (stage II), ripe (stage III).

### 178 **2.4 Biomarkers measurement**

179 At T12, 15 animals per group were sampled for biomarker analyses. All the analyses except  
180 metallothioneins were performed on the same individuals. For genotoxicity, the comet assay and  
181 the micronuclei test were performed on mussel hemocytes extracted from the adductor muscle.  
182 The comet assay was performed as previously described (Revel et al., 2019). Following sampling,  
183 individual hemocytes were recovered by centrifugation (1500 rpm, 5 min) of the hemolymph  
184 samples. The hemocyte pellet was then resuspended in 160  $\mu$ L of 0.5% low melting point agarose  
185 for the preparation of 2 comet slides. Following lysis and denaturation in the electrophoresis  
186 buffer, DNA migration was performed for 15 min at 23 V (390 mA, E = 0.66V cm<sup>-1</sup>). At the end of

187 electrophoresis, the slides were washed by incubation for  $3 \times 5$  min in Tris base 0.4 M, pH 7.5. In  
188 order to obtain permanent preparations, the slides were immersed in absolute ethanol for 10 min  
189 to dehydrate, then dried at room temperature. Just prior to analysis, 75  $\mu\text{L}$  of GelRed at  $8 \text{ mg L}^{-1}$   
190 were spread over each slide using a cover glass. The slides were placed for at least 1 h in the dark at  
191  $4^\circ\text{C}$  for coloration, then analyzed using an optical epifluorescence microscope (Olympus BX60,  $\times 40$ )  
192 equipped with a CDD camera (Luca-S, Andor Technology) and image analysis system (Komet 6,  
193 Kinetic Imaging Ltd.). At least 50 nuclei were analyzed per slide and the percentage of DNA present  
194 in the Comet tail (% Tail DNA) was measured for each observed nucleus. For micronuclei, 100  $\mu\text{L}$  of  
195 hemolymph were deposited on a poly-L-Lysine slide. After incubation for 20 min in a humid  
196 chamber, the hemocytes were fixed with ethanol/acetic acid solution (3V/1V) for 20 min and then  
197 rinsed twice in PBS. The nuclei of the cells were then stained with DAPI ( $1 \mu\text{g} \cdot \text{mL}^{-1}$ ) for 15 min, then  
198 placed between the slide and coverslip with Mowiol mounting liquid. A semi-automated count of  
199 micronuclei on 1000 cells per individual was performed on CellInsight CX5 HCS<sup>®</sup> platform (Thermo).  
200 For acetylcholinesterase activity (AChE), gills were dissected and frozen in liquid nitrogen until  
201 analyses. The method described by Ellman et al. (1961) was used, adapted for 96-microplate reader  
202 (Bocquéné G., 1998). The activity was measured in S9 fractions from mussel gills. The protein  
203 content was determined for each sample according to the Bradford method using BioRad Protein  
204 Assay Dye Reagent; bovine serum albumin was used as standard. Each sample was measured in  
205 triplicate and absorbance read at 595 nm. One hundred  $\mu\text{g}$  of protein (S9 fraction at  $1 \mu\text{g} \cdot \mu\text{L}^{-1}$ ) were  
206 added to 250  $\mu\text{L}$  of 2 mM sodium phosphate buffer (pH 7, containing 0.1% of TritonX-10); then 20  
207  $\mu\text{L}$  of dithioisnitrobenzoate 10 mM (Sigma – Ref D8130) and 10  $\mu\text{L}$  of acetylthiocholine 100 mM  
208 (Sigma – Ref D5751) were added. AChE activity was measured by reading the optical density on a  
209 microplate reader (Safire TECAN) at 412 nm after 2 min incubation at room temperature under  
210 gentle shaking. Results are given as  $\text{units} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ .

211 For lysosomal biomarkers, digestive glands were dissected out and frozen in liquid nitrogen until  
212 analyses. The lysosomal membrane stability (LMS) was determined in serial cryotome sections (10  
213  $\mu\text{m}$ ) of mussel digestive gland after demonstration of hexosaminidase (Hex) activity, according to  
214 UNEP/RAMOGE (1999). The time of acid labilisation treatment required to produce the maximum  
215 staining intensity was assessed under a light microscope and was denoted as the Labilisation Period  
216 (LP; in min). Four determinations were made per individual; for each area, the first maximum  
217 staining peak was considered to determine the LP value (Martínez-Gómez et al., 2015). A final LP  
218 value was calculated for each individual as the mean of the four LP values determined in each area.

219 For Lysosomal structural changes (LSC), the visualisation of lysosomes in digestive cells of *Mytilus*  
220 sp. was based on the histochemical demonstration of  $\beta$ -glucuronidase activity, according to the  
221 procedure described by Moore (1976) with the modifications made by Cajaraville et al. (1989). The



222 structure of lysosomes was assessed through a stereological procedure based on image analysis  
223 (BMS, Sevisan) according to Cajaraville et al. (1991). Five measurements using a 100x objective lens  
224 were made per individual. The mean value of the following stereological parameters was  
225 determined for each mussel digestive gland (Lowe et al., 1981): lysosomal volume density ( $V_{V_{LYS}}$ );  
226 lysosomal surface to volume ratio ( $S/V_{LYS}$ ) and lysosomal numerical density ( $NV_{LYS}$ ). Intracellular  
227 neutral lipids were histochemically demonstrated in crytome sections (8  $\mu\text{m}$ ) of mussel digestive  
228 glands stained with Oil Red O (ORO) (Culling 1974). Neutral lipid volume density ( $V_{V_{NL}}$ ) was  
229 determined through a stereological procedure using image analysis (BMS, Sevisan) by  
230 measurement of five areas per individual, using a 40x objective lens.  
231 Metallothioneins (MTs) concentrations were analyzed on the full animal flesh (6 replicates per  
232 group) with the indirect method of inorganic mercury saturation as described by Baudrimont et al.  
233 (2003). For this measure, flesh was immediately placed in liquid nitrogen until measurement. The  
234 device used to measure the mercury is an atomic absorption spectrometer by heat treatment  
235 under oxygen flow (AMA 254, SYMALAB), whose limit of detection is 1 ng of mercury.

## 236 **2.5 Chemical analyses**

237 At T5 and T12, mussels were sampled according to the ROCCH protocol (ROCCH, 2016) adapted  
238 from (CEMP/OSPAR, 2018) consisting in purifying the animals for at least 18 hours in seawater to  
239 minimize the contamination coming from gut content. Mussels were shelled and their flesh drained  
240 on a porcelain Buchner funnel before being placed in pre-combusted glass containers and stored at  
241  $-20^{\circ}\text{C}$ . A minimum of 30 mussels per population were sampled to obtain one and unique  
242 representative pool per group used for isotope and chemical analysis. The samples were then  
243 lyophilized and grounded using a zirconium oxide ball mill to obtain a fine and homogeneous  
244 powder compatible for all analyses.

### 245 **2.5.1 Carbon and nitrogen stable isotope analyses**

246 Aliquots of homogenised powder ( $0.40 \pm 0.05$  mg) were weighed in tin cups. Analyses were  
247 performed with a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Thermo  
248 Scientific Flash EA1112 elemental analyser. The results are presented in the usual  $\delta$  notation  
249 relative to the deviation from international standards (Pee Dee Belemnite for  $\delta^{13}\text{C}$  values, and  
250 atmospheric nitrogen for  $\delta^{15}\text{N}$  values), in parts per thousand (‰). Based on replicate  
251 measurements of internal laboratory standards (acetanilide), the experimental precision was  
252  $<0.15\text{‰}$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Variations in organisms' lipid content may affect  $\delta^{13}\text{C}$  values, while  
253 food sources do not change. This is because lipids are considerably depleted in  $^{13}\text{C}$  compared to  
254 other tissue components (DeNiro and Epstein, 1977). Moreover, lipid content can vary greatly  
255 according to the season in bivalves, as shown by seasonal variations in their C/N ratio (i.e. proxy of

256 lipid content; Briant et al., 2018). In order to compare isotopic compositions of bivalves over the  
257 study period,  $\delta^{13}\text{C}$  values were thus corrected according to the formulae of (Post et al., 2007).

### 258 **2.5.2 Trace metal analyses**

259 Total mercury (Hg) in mussels was assessed by atomic absorption spectrophotometry on aliquots of  
260 homogenised powder ( $50 \pm 5$  mg), using an Advanced Mercury Analyser (ALTEC AMA-254, Altec  
261 Ltd) and according to the standard operating procedure described in the US-EPA method N°7473  
262 (U.S. Environmental Protection Agency, 1998). The limit of quantification (LOQ) was  $0.015 \text{ mg kg}^{-1}$   
263 dry weight (dw). Total chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), silver (Ag), cadmium (Cd)  
264 and lead (Pb) concentrations were determined with a Quadrupole Inductively Coupled Plasma Mass  
265 Spectrometer (Q-ICP-MS, ICAP-Qc model from ThermoFisher), according to an in-laboratory  
266 approved method. Briefly, aliquots of samples (~200 mg of homogenised powder) were placed in  
267 Teflon bombs and mineralized with a mixture of ultrapure  $\text{HNO}_3$  acid and ultra-pure water using a  
268 microwave digestion system (ETHOS-UP model from Milestone). The digests were then diluted to  
269 50 mL with ultra-pure water before analyses with Q-ICP-MS. With this method, LOQs (in  $\text{mg kg}^{-1}$   
270 dw) were 0.12 Cr, 0.03 for Ni, 0.11 for Cu, 1.2 for Zn, 0.01 for Ag and Cd, and 0.08 for Pb. The  
271 quality assurance of all metal analyses relied on blank and internal standard controls, and on the  
272 accuracy and reproducibility of data relatively to the certified reference materials (CRMs) used for  
273 analytical runs. Blank values were systematically below the detection limits and CRM values  
274 concurred with certified concentrations, with recovery rates ranging between 85% and 101%. The  
275 CRMs used were IAEA-142 (mussel homogenate; International Atomic Energy Agency) for Hg, and  
276 CE-278k (mussel tissue, Joint Research Centre- European Commission) and SRM-1566b (oyster  
277 tissue, National Institute of Standards and Technology) for the other trace metals.

### 278 **2.5.2 Organic contaminants measurement**

279 For PCB, PBDE, and lipid content, ground freeze-dried tissues (1 g) were extracted by pressurized  
280 liquid extraction (PLE - SpeedExtractor - Buchi, France) with a mixture of toluene/acetone (70/30).  
281 The extracts were sequentially cleaned-up on acidified silica and florisil. PCB (dioxin-like: 105, 114,  
282 118, 123, 156, 157, 167, 189; and non-dioxin-like: 28, 52, 101, 138, 153, 180) and PBDE (28, 47, 99,  
283 100, 153, 154, 183) congeners were screened in the final extract by Gas Chromatography – High  
284 Resolution Mass Spectrometry (GC-HRMS – magnetic sector). Quantification was performed by  
285 isotopic dilution using the  $^{13}\text{C}$ -labelled homologue of each of the analytes. For PAH analysis, freeze-  
286 dried tissues (0.3 g) were extracted by a mixture of hexane/acetone (50/50) in a PLE system and the  
287 extracts were further purified on a styrene-divinylbenzene copolymer sorbent. Analysis was  
288 performed on a gas chromatograph coupled with a tandem quadrupole mass spectrometer (GC-  
289 MS/MS) and PAHs were quantified against their D- or  $^{13}\text{C}$ -labelled homologue. For all classes of

290 organic contaminant and in each set of 10 samples, a blank sample and a quality control were also  
291 analyzed. At least three technical replicates were performed for all analyses.

## 292 **2.6 Statistics**

293 The R software was used for the statistical analysis of the data. Normality and homoscedasticity of  
294 the data were then tested on residues with the Shapiro-Wilk and Fligner-Killeen tests respectively.  
295 The differences between experimental groups were tested by one-way ANOVA or Kruskal-Wallis  
296 test followed by Fisher's Least Significant Difference or Dunn's test, respectively, for post hoc tests;  
297  $p$ -values  $< 0.05$  were considered as significant. Correlations tests were performed using the  
298 Pearson's product moment correlation coefficient on the average percentage of *M. edulis* alleles  
299 (on the 3 nuclear markers described in 2.2 paragraph) and ecotoxicology biomarkers (Comet test,  
300 micronuclei, LMS, AChE).

301

## 302 **3 Results**

### 303 **3.1 Genetic and physiological parameters**

304 Genetic markers on progenies globally confirmed the multimarker approach used to genotype the  
305 parents (Appendix B). WIM and BIA groups were confirmed to be mostly pure *M. edulis* (on average  
306 77% homozygotes) and *M. galloprovincialis* (on average 78% homozygotes) respectively. MOG  
307 progenies were on average 51% homozygotes for *M. galloprovincialis* with many individuals being  
308 heterozygotes (45%). YEU progenies showed almost half individuals (44%) being heterozygotes for  
309 the three genetic markers.

310 At T0 and T5, no differences in shell length between the four groups were registered. At T12, shell  
311 length was  $38 \pm 5$  mm for YEU,  $31 \pm 3$  mm for WIM,  $30 \pm 4$  mm for BIA and  $28 \pm 3$  mm for BIA. For  
312 both shell length and Meat yield significant differences were found between the groups ( $p$ -value  $<$   
313 0.001), the YEU group showing the highest values and BIA the lowest ones of Meat yield (Figure 1).  
314 The cumulative mortality at T12 was significantly different ( $p < 0.0001$ ) among the groups, ranging  
315 from WIM 10%  $<$  YEU 23%  $<$  BIA 29%  $<$  MOG 31%.

316 At T12, the four mussel groups presented different stages of gonad development (Figure 2). The BIA  
317 group showed the more limited development of their gonads, restricted the outer layer of the duct.  
318 Very few germinal cells were observed for mussels of this group. The WIM and MOG mussels  
319 presented only the early stages of gametogenesis. The aspect of the storage tissue in these groups  
320 was different, and more developed in the WIM group. The YEU group presented heterogeneous  
321 individuals in term of gonad development, from quiescent stage to developing meiosis stage. No  
322 sexually mature mussel was observed in any group studied.

### 323 **3.2 Ecotoxicological biomarkers**

324 Average DNA strand breaks were  $8.3 \pm 2.1\%$  (min = 4.1%, max = 13.5%), no significant differences  
325 were found between groups (Figure 3a). The micronuclei test showed levels comprised between  
326 0‰ and 3‰, and did not show any significant differences between the four mussel groups (Figure  
327 3b). Acetylcholinesterase activity (AChE) showed overall mean levels of 103 nmol/min/mg prot and  
328 differences between groups ( $p$ -value = 0.015) with lower levels for BIA group (Figure 3c).  
329 Metallothionein capacity showed higher levels for the WIM group ( $p$ -value = 0.032, Figure 3d). In  
330 lysosome membrane stability (LMS) test, the mean lysosomal period (LP) was  $19.0 \pm 1.2$  min. The  
331 highest LP values were recorded in *M. galloprovincialis* (BIA:  $22.9 \pm 1.2$  min and MOG:  $21.6 \pm 3.7$   
332 min) and the lowest values were recorded in *M. edulis* (WIM  $16.0 \pm 2.3$  min) and in hybrids mussels  
333 from YEU ( $15.5 \pm 1.0$  min) ( $p$ -value = 0.002, Figure 3e).

334 Overall, lysosomes in *M. galloprovincialis* from BIA and MOG were small and close to detection limit  
335 (Appendix C); on the contrary, WIM and YEU showed larger lysosomes. Accordingly, WIM and YEU  
336 were characterised by significantly higher  $Vv_{LYS}$  and lower  $S/V_{LYS}$  (inverse to size) than BIA and MOG.  
337 Additionally, significantly higher  $Nv_{LYS}$  was detected in WIM. The volume density of intracellular  
338 neutral lipids ( $Vv_{NL}$ ) recorded in digestive cells of mussel ranged from  $0.0006 \pm 0.0004 \mu\text{m}^3/\mu\text{m}^3$   
339 (BIA) to  $0.04 \pm 0.009 \mu\text{m}^3/\mu\text{m}^3$  (YEU).  $Vv_{NL}$  was significantly higher in mussels from YEU than in the  
340 other experimental groups.  $Vv_{NL}$  from WIM was significantly higher than  $Vv_{NL}$  measured in MOG.

341 As genetic markers and biomarkers were performed on the same individuals, correlations tests  
342 were performed. *M. edulis* alleles were positively correlated to Comet test results ( $p$ -value = 0.04,  
343 cor 0.27) and AChE ( $p$ -value = 0.002, cor 0.39) and negatively correlated to LMS ( $p$ -value = 0.03, cor  
344 -0.28).

### 345 **3.3 Isotope analyses**

346 Compared to the signing of spat at T0, the mussel progenies at T5 had slightly higher values of  $\delta^{13}\text{C}$   
347 and  $\delta^{15}\text{N}$ . At T12, higher  $\delta^{13}\text{C}$  values were observed compared to T5, while  $\delta^{15}\text{N}$  values remained  
348 very similar between the 2 sampling periods. In terms of variations between progenies at T12,  
349 WIM, BIA and MOG progenies showed almost identical signatures in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , while YEU had a  
350 very different isotopic composition, confirmed by replicate analyses (Figure 4).

### 351 **3.4 Trace metal analyses**

352 As a general trend, after 5-month field transplantation, concentrations were higher (between 2 and  
353 10 times higher) than after one year in the field (T12), for all the trace metals studied (Figure 5). At  
354 T12, for essential trace elements (Cr, Ni, Cu, Zn), trends between mussels progenies were observed  
355 for Zn (WIM < BIA), and to a lesser extent for Cu (reverse trend WIM > BIA). In addition, MOG group  
356 had similar contents than BIA (with the exception of Cu), while YEU batch was generally at the same  
357 level as WIM or had lower concentrations than all other progenies.

358 For non-essential trace elements (Ag, Cd, Hg, Pb), a marked trend for Cd was observed (with WIM  
359 <BIA), and to a lesser extent for Hg (similar trend WIM <BIA). In addition, as with essential metal  
360 trace elements, MOG had concentrations equivalent to BIA, while YEU was generally at the same  
361 level as WIM or had lower concentrations than all the other progenies.

### 362 **3.5 Organic contaminants**

363 Percentage of lipid content were  $8.9 \pm 1.7\%$  in MOG, followed by WIM  $8.5 \pm 0.5\%$ , BIA  $8.4 \pm 1.1\%$   
364 and YEU  $6.4 \pm 0.5\%$ . The values measured for PCBs, PBDEs and PAHs are presented in Appendix D.  
365 The PCB profiles were similar regardless of the considered progeny, allowing the use of the most  
366 abundant PCB congener (CB 153) for group comparison (Figure 6a). PCBs were measured at T0 in  
367 the nursery at similar levels for all progenies, CB 153 being comprised between 4.7 and 6.1  $\text{ng.g}^{-1}$   
368 dw. At T5, the PCB levels remained similar between progenies compared to T0, with no increase  
369 (CB 153 between 4.7 and 5.6  $\text{ng.g}^{-1}$  dw). At T12, MOG and BIA progenies had concentrations of CB  
370 153 37 to 47% lower than and YEU, while WIM was only 14% lower.

371 Regarding PBDEs, the general profiles of congeners were also similar for all progenies with a  
372 predominance of BDE 47, 99 and 100 (Figure 6b), in agreement with previously published (Munschy  
373 et al., 2015). For all congeners, the highest levels were measured at T0 in the nursery with  
374 concentrations of BDE 47 between 0.22 and 0.25  $\text{ng.g}^{-1}$  dw, decreasing to 60% and 35% of this value  
375 at T5 and T12 respectively. At T12, trends in concentrations between the mussel progenies were  
376 observed, BDE 47 concentrations of BIA and YEU being both 47 and 83% lower than MOG and WIM,  
377 respectively. The 99/100 PBDE congener ratio (Figure 6b), used as a proxy of metabolic capacities  
378 (e.g. in fish Munschy et al., 2011), was lower for WIM and higher for BIA at T12, by a factor of more  
379 than 2. For PAHs, higher concentrations (sum of the 14 PAHs measured) were observed at T5 and  
380 then decreased at T12 when compared to T0. Ratio of pyrene to the less metabolizable  
381 fluoranthene, proxy of metabolic capacities, was higher for MOG progeny.

382

## 383 **4 Discussion**

### 384 **Physiological parameters**

385 The aim of this study was to investigate whether mussel genetics could affect physiological  
386 parameters, ecotoxicological biomarker and contaminant bioaccumulation. Progenies of 4 mussel  
387 populations presenting different genetic backgrounds, produced and grown under the same  
388 conditions were transplanted *in situ* and showed significant differences on physiological traits such  
389 as growth and mortality. As already observed in a laboratory experiment following copper  
390 exposure, Meat yield was lower for *M. galloprovincialis* compared to *M. edulis* (Brooks et al., 2015).  
391 Moreover here, the highest Meat yield was observed for the YEU group (mussel progeny from the

392 Ile d'Yeu) originating from a hybrid zone including individuals from both species, introgressed and  
393 hybrids individuals. Given the presence of hybrid individuals in this group, these results could be  
394 linked to the concept of heterosis (hybrid vigour), already demonstrated in *M. edulis*/*M.*  
395 *galloprovincialis* hybrids (Bierne et al., 2002), hybrids having the ability to faster adapt to a large  
396 range of new conditions. Differences in Meat yield or condition index are related to different  
397 growth rates between species. Indeed age to size correlations vary not only from geographical  
398 areas but also depending on the studied species (Izagirre et al., 2014). Age differences for  
399 individuals of the same size were related to differences in contaminant bioaccumulation between  
400 *M. edulis* and *M. trossulus* (Lobel et al., 1990), and might induce bias in biomonitoring observations  
401 (i.e. older mussel presenting higher contaminant levels). To solve this bias, (Izagirre et al., 2014)  
402 recommended mussel age standardization in mussel biomonitoring program. Concerning mortality,  
403 in the present study *M. galloprovincialis* mussels (BIA *M. galloprovincialis* progeny from Basque  
404 Coast and MOG *M. galloprovincialis* progeny from Brittany) had higher mortality rates than the  
405 other species. These same populations were tested on several other sites in the framework of the  
406 Ifremer MORBLEU projects (Pepin et al., 2019), and significant population mortality was found by  
407 site interaction. MORBLEU data showed that during episodes of abnormal mortality outbreaks, *M.*  
408 *edulis* was more sensitive than *M. galloprovincialis*, their hybrids being intermediates (Pepin et al.,  
409 2019). However, in the absence of abnormal mortalities, *M. galloprovincialis* seems less adapted to  
410 the Marennes-Oléron Bay conditions compared to *M. edulis*, which is linked with the distribution of  
411 *Mytilus* species along the French Atlantic coast, with *M. galloprovincialis* North and South of the  
412 Marennes-Oléron Bay and Charentais. In our study, sound differences on gametogenesis stages  
413 seemed to subsist among the four mussel populations. In the annual cycle of *M. edulis*, gonads start  
414 to redevelop in November to become fully mature in early spring (Seed, 1975). Furthermore, the  
415 gametogenesis cycles of the two species seem to vary within the same site (Bayne, 1976), and an *in*  
416 *situ* study showed that populations of *M. edulis* and *M. galloprovincialis* coexisting in southern  
417 England had a gametogenesis and egg-laying lag of around eight weeks, *M. galloprovincialis*  
418 reproducing later than *M. edulis* (Seed, 1971). This seems consistent with our results, the WIM  
419 group (*M. edulis* progeny from North of France) being in gametogenesis stages I or II, while BIA  
420 being mainly at stage 0 and I. Here the YEU progeny showed a greater heterogeneity in  
421 gametogenesis stages, which might reflect its genetic heterogeneity. Moreover, YEU had more  
422 individuals at gametogenesis stage II, potentially linked with the higher Meat yield observed, or a  
423 better adaptability to the local conditions of the transplantation site. These results also concern  
424 only 10 individuals per progeny, which represents a relatively small number in order to be able to  
425 draw general conclusions on the different genotypes. In a biomonitoring perspective and according  
426 to CEMP/OSPAR (2018), biomonitoring should be performed out of the active spawning season

427 (spawning peaks are in springtime and autumn, when individuals may lose up to 50% of their soft  
428 tissue weight). Here if the differences in Meat yield observed stay comparable between groups in  
429 November outside of the active spawning season, differences in the gametogenesis stages are  
430 found between *Mytilus* species, potentially leading to bias in flesh lipid contents directly influencing  
431 contaminant affinity to tissues.

#### 432 ***Ecotoxicology biomarkers***

433 Concerning genotoxicity, in a previous laboratory study, micronucleus formation in *M. edulis* was  
434 higher at basal laboratory conditions compared to *M. galloprovincialis* (Brooks et al., 2015), but in  
435 our study, the different progenies showed similar genotoxicity biomarker levels. Genotoxicity data  
436 observed here were lower than Background Assessment Criteria (BAC) established for micronuclei  
437 (*M. edulis* BAC=2.5‰; *M. galloprovincialis* from the Mediterranean Sea BAC=3.9‰, Davies and  
438 Vethaak, 2012), and for DNA strand breaks (*M. edulis* BAC=10%, Davies and Vethaak, 2012),  
439 indicating that mussels did not undergo genotoxic stress. In our study, AChE activity was lower in  
440 BIA progeny originating from Basque Coast. Environmental Assessment Criteria (EAC) indicating  
441 that organisms are exposed to chemical stress are 20 and 21 nmol/min/mg prot for *M. edulis* and  
442 *M. galloprovincialis* respectively (AChE assay measures an enzymatic inhibition, indeed higher levels  
443 indicate a good environmental status, Davies and Vethaak, 2012). AChE activity levels measured  
444 here are all above the EAC, suggesting that mussels were not under a neurotoxic stress important  
445 enough to inhibit AChE activity.

446 Separately, we observed a higher lysosomal membrane stability for BIA *M. galloprovincialis* than in  
447 the other groups, whereas in laboratory conditions no differences were observed between species  
448 (Brooks et al., 2015). In general, in mussel, values above 20 min are considered characteristic of  
449 healthy individuals (BAC, Davies and Vethaak, 2012), whilst values 10 min < LMS < 20 min indicate  
450 stressed but compensating animals (Davies and Vethaak, 2012). Thus, general health status of  
451 mussel analysed in the present experiment varied between groups, indicating slight biological  
452 effects of environmental stressors in the case of *M. edulis* (WIM) and hybrid mussels from YEU.  
453 Similarly, the lysosomal structure assessed by the LSC test varied between experimental groups.  
454 Larger lysosomes were observed in *M. edulis* (WIM) and hybrid mussels from YEU than in *M.*  
455 *galloprovincialis* from BIA and MOG. Changes in intracellular accumulation of neutral lipids in  
456 digestive cells of mussel have been recorded as indicators of exposure to contaminants and  
457 especially organic compounds. In the present study, higher levels of V<sub>VNL</sub> were detected in hybrid  
458 mussels from YEU and to a lesser extent in *M. edulis* from WIM. It is worth mentioning that this  
459 parameter may be subjected to seasonal variations in relation with the reproductive cycle (Cancio  
460 et al., 1999). Overall, in this experiment, lysosomal biomarkers indicate a good general health  
461 status recorded in *M. galloprovincialis* from BIA and MOG and a slight stress recorded in *M. edulis*

462 (WIM) and in hybrid mussel (YEU) which could indicate a higher sensitivity of these groups. Here,  
463 BAC levels were higher in *M. edulis* from WIM suggesting a higher responsiveness of the species, as  
464 suggested by lysosomal biomarkers.

465 Even if performed only on a limited panel of nuclear markers, here the correlation tests results  
466 confirms that individuals with more *M. edulis* alleles seems more sensitive to general stress (higher  
467 values for % Tail DNA in the Comet test and lower levels of LMS) but have better anti-neurotoxicity  
468 capabilities (higher AChE levels). Anyway, to generalize such results to a specie or genetic  
469 background more investigation are need using more populations and more locations.

#### 470 **Chemical analysis**

471 Isotope analyses showed variations in  $\delta^{13}\text{C}$  in particular between T5 and T12 for all progenies. This  
472 can be linked to: i) the preferential excretion of  $^{12}\text{C}$  compared to  $^{13}\text{C}$  in metabolic processes (e.g.  
473 respiration) in consumers (De Niro and Epstein, 1978), which generates a natural enrichment in  $^{13}\text{C}$   
474 with age regardless of the diet; ii) potential differences in terms of filtration capacity between  
475 young and older mussels (Sukhotin et al., 2003), and potential differences in the assimilation of  
476 trophic sources; iii) potential differences in the trophic sources available for mussels between T5  
477 and T12, linked to the seasonal features. At T12, variations were also observed for the YEU batch  
478 compared to other batches, for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. This suggests different assimilated  
479 trophic sources for YEU mussels, and/or different biological fractionation of C and N due to  
480 metabolism for this progeny (in the case where similar trophic sources than the other batches had  
481 been ingested).

482 Regarding trace metals, the higher concentrations observed at T5 compared to T12 for most metals  
483 may be first related to biodilution due to mussel body mass gain, and/or to lower metal intake over  
484 time. Then, at T12, the YEU group had the highest Meat yield and the lowest concentrations of  
485 trace metal elements (except for Zn), suggesting a more pronounced metal biodilution in the YEU  
486 group than in the other ones. Decreased concentrations in several ETM (i.e. Cd, Co, Cr, Cu, Ni, Pb  
487 and Zn) concomitantly with increase in body weight and size was indeed reported in *M. edulis*  
488 (Mubiana et al., 2006), further highlighting the importance of collecting mussels of similar  
489 size/body weight/condition index when sampling for biomonitoring programs (CEMP/OSPAR, 2018).  
490 At T12, YEU also had very distinct C and N compositions from other groups. As the trophic path  
491 remains a major source of exposure and incorporation of trace metals in soft tissues of bivalves  
492 (Fisher et al., 1996; Wang et al., 1996; Metian et al., 2009), the potentially different trophic sources  
493 assimilated by YEU could also explain the observed variations in terms of metal bioaccumulation for  
494 this progeny.

495 Trace metal regulation mechanisms also seem to be different in the four progenies, as WIM batch  
496 presents at T12 the highest metallothionein (MTs) levels and is also the one that has lower Zn, Cd



497 and Hg concentrations than BIA and MOG, but higher Cu concentrations compared to BIA (Figure  
498 7). Interestingly, other studies found higher MT concentrations in *M. galloprovincialis* compared to  
499 *M. edulis*, and in the first species MTs induction seemed to depend on several metals, while in *M.*  
500 *edulis* essentially on Cd (Amiard et al., 2006; Zorita et al., 2007). In marine organisms and especially  
501 in bivalves, MTs are involved in the homeostasis of essential trace elements such as Cu and Zn, but  
502 also in the detoxification of certain non-essential metal trace elements such as Cd, Ag or Hg. MTs  
503 complex these ions and store them in an inert form, or even allow their elimination (Amiard et al.,  
504 2006). Thus, higher MTs activity/concentration would be induced during a significant exposure of  
505 organisms to trace metals, but it may also be induced by other environmental stresses such as an  
506 increase in temperature (Le et al., 2016; Wang and Rainbow, 2010). On the other hand, MTs activity  
507 might also be constitutionally higher in some species, explaining lower levels of metal trace  
508 elements (Amiard et al., 2006). Therefore, the processes involved in these mechanisms are still  
509 poorly understood, and the significance of high levels of MTs measured in organisms is still subject  
510 of debate.

511 Here, it is likely that in YEU mussels the lower Zn, Cd and Hg concentrations and higher Cu  
512 concentrations are explained by differentially compensation of the metabolic process. Concerning  
513 Cu bioaccumulation, *M. edulis* was found to have higher levels after a 21 days experimental  
514 exposure to copper compared to *M. galloprovincialis* (Brooks et al., 2015). In our study WIM and  
515 BIA progenies follow the same trend (*M. edulis* > *M. galloprovincialis*), but MOG group (*M.*  
516 *galloprovincialis* introgressed with *M. edulis*) had the highest Cu content (Figure 7), indicating that  
517 interpretations of bioaccumulation variations in mussel species are further complicated by  
518 introgression and hybridization phenomena. Finally, the results obtained demonstrate  
519 bioaccumulation variations between mussel species for certain metal trace elements such as Zn,  
520 and to a lesser extent Cd, Hg and Cu, which deserves to be confirmed on other transplantation sites  
521 or by laboratory experiments.

522 PCB concentrations varied between progenies, with higher values in YEU and WIM (hybrid zone and  
523 *M. edulis*), compared to BIA and MOG (mostly *M. galloprovincialis*). These trends were observed in  
524 both dry weight and lipid weight normalized concentrations, suggesting they did not relate to the  
525 tissue's biochemical composition. They could eventually be attributed to (i) a higher filtration rate,  
526 and therefore a higher exposure to environment contamination or (ii) a more effective elimination  
527 process in other groups. Furthermore, YEU progeny was the one with highest Meat yield, to be  
528 potentially linked with higher filtration rates. Unfortunately, data on differential filtration rates and  
529 metabolization processes between these two species are lacking to fully explain these results. As  
530 for metal trace elements, lower BDEs concentrations are observed for T5 and T12 compared to T0,  
531 indicating biotransformation and excretion and / or biodilution by growth. Interestingly, since

532 biota exposure pathways are similar for PBDEs and PCBs, this BDE-specific decrease seems to  
533 indicate elimination rather than biodilution which would also have affected PCBs. In our study BDE  
534 concentrations were higher in MOG and WIM progenies, making difficult to draw any conclusion on  
535 potential genetic effects. Studies on fish showed that the penta-BDE congeners 99 and 100 do not  
536 have the same susceptibility to biotransformation since congener 99 is metabolized by  
537 debromination when BDE 100 is not, despite their similar physico-chemical properties (Munsch et  
538 al., 2011). First order kinetics of relatively comparable constants were found in mussels for both  
539 PCBs and PBDEs in lab conditions (Gustafsson et al., 1999). However the strength of our study is to  
540 be under real environmental conditions in the marine environment. Unlike lab-based experiments,  
541 phytoplankton and particle contaminations are not characterized and are expected to vary  
542 significantly. As such, interpretation of results against uptake/metabolisation/depuration kinetics  
543 requires caution. If this approach remains valid for bivalves, the PBDE 99/100 congener *ratio* (6b)  
544 can therefore be an indicator of metabolic capacities, where high values would indicate low  
545 metabolic capacities. At T12, this ratio is less important for WIM suggesting higher metabolic  
546 capacities for *M. edulis*. Using a similar approach, no difference was found between progenies on  
547 PCB congeners of different susceptibility to metabolization in fish and mammals (Boon et al., 1997;  
548 Buckman et al., 2006). Concerning PAHs, concentrations increased at T5 and lowered at T12  
549 indicating different accumulation / metabolization over time as for BDE. If at T12 no variations  
550 could be observed for the sum of PAHs between the four progenies, pyrene/fluorantene ratio, also  
551 indicating metabolization capacities (Arnot et al., 2008), showed lower levels for WIM, also  
552 suggesting a better metabolization capacity for PAHs in this progeny as already suggested for BDEs.

553

## 554 **Conclusion**

555 Overall these results indicate that the contaminant bioaccumulation in *Mytilus* sp. differ depending  
556 on the species. Furthermore, differences in physiological parameters and ecotoxicological  
557 biomarkers were observed between transplanted groups. However, no specific trend could be  
558 identified concerning the capacity of one or the other species to bioaccumulate more or less  
559 chemical contaminants. The patterns of interspecific variations observed varied according to the  
560 contaminants targeted. After 12 months on site, trends in contamination levels were observed  
561 between progenies for most contaminants, with higher levels of PCB, PBDE and Cu in *M. edulis*, and  
562 higher levels of Zn, Cd, Hg for *M. galloprovincialis*. Contaminant elimination mechanisms through  
563 either regulation (MTs - trace metals) or metabolization (PBDEs, PAHs) seemed also to be more  
564 efficient in *M. edulis*.

565 The results obtained have implications for biomonitoring programs, and would benefit from further  
566 studies on other mussel with different genetic background, and on a longer time scale, with the

567 analysis of individuals of the same age/size (and sampled in the same season) as those of  
568 biomonitoring programs (i.e. for ROCCH program: 40 mm mussel sampled in February). In  
569 particular, it will be interesting to assess changes in magnitude of biological responses to  
570 contaminants in a future study on sites having different pollution status. In addition, a better  
571 understanding of the physiology of these two species, their depuration and metabolism capabilities  
572 would help understanding the observed differences. The consequences on the interpretation of  
573 biomonitoring data from mussels are difficult to predict to date, but taking into account the genetic  
574 parameter in databases seems judicious.

575

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583

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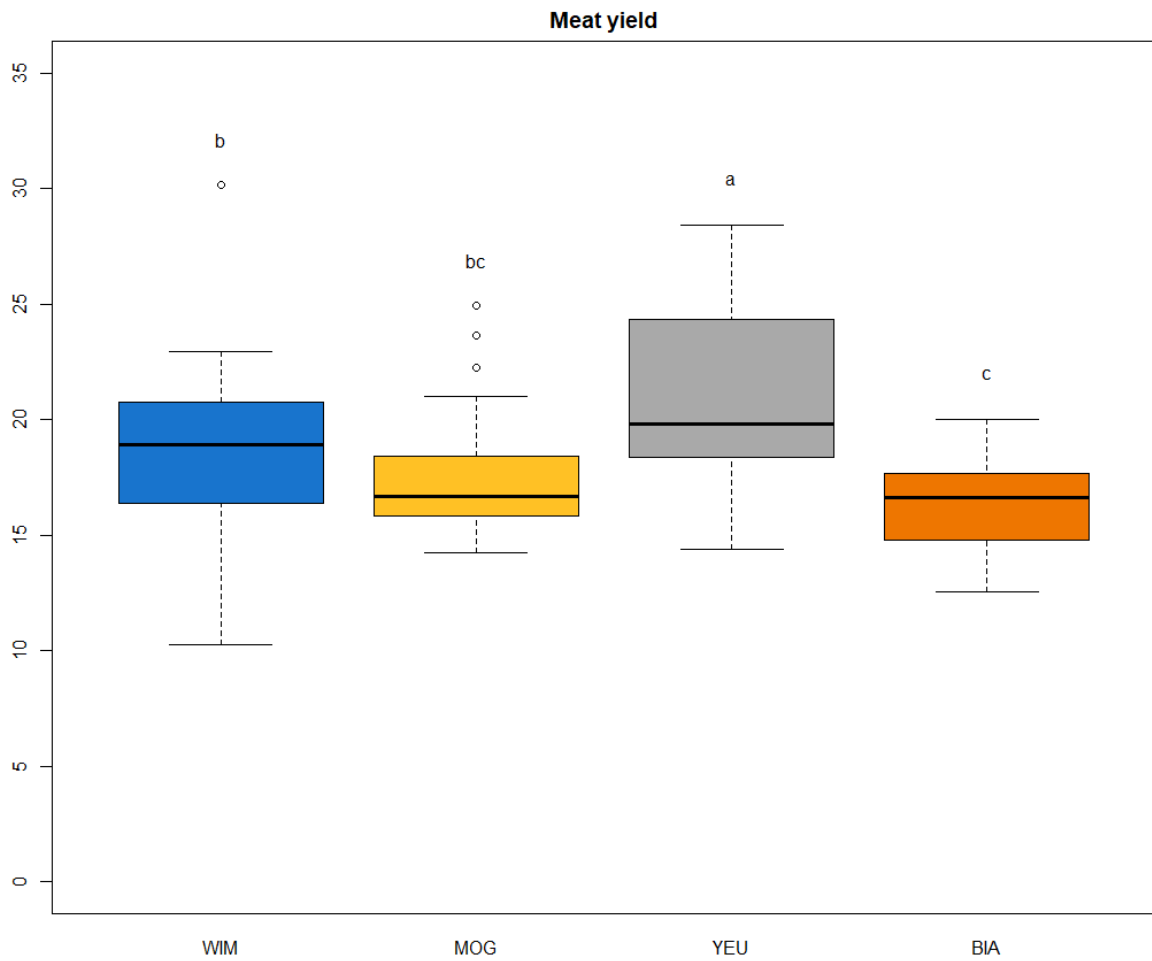
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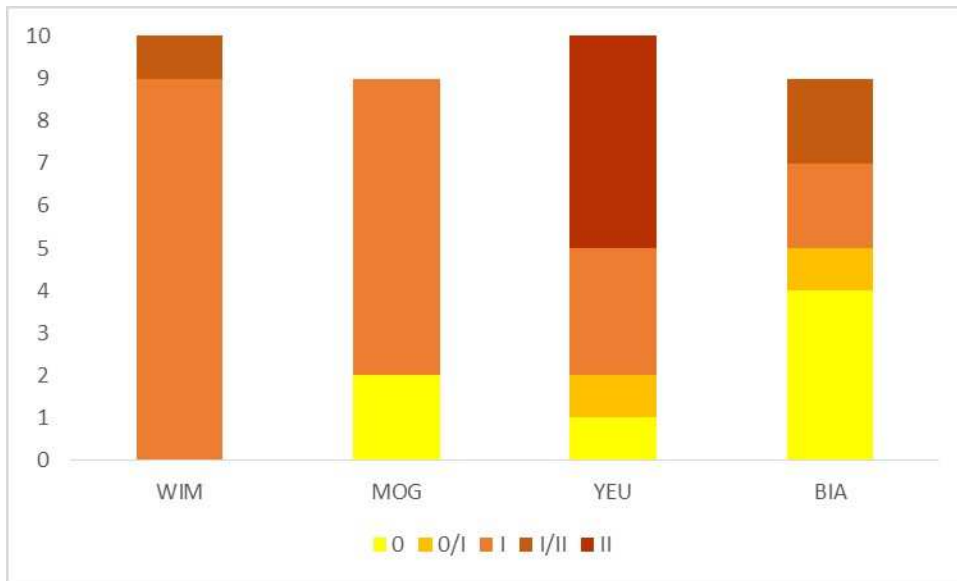


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769 Figure 1. Meat yield of the four mussel progenies at the end of the field experimentation (T12) (WIM,  
 770 Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M.*  
 771 *edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*). Letters  
 772 represent statistically different groups calculated by the Fisher's Least Significant Difference post hoc test  
 773 (n=30).

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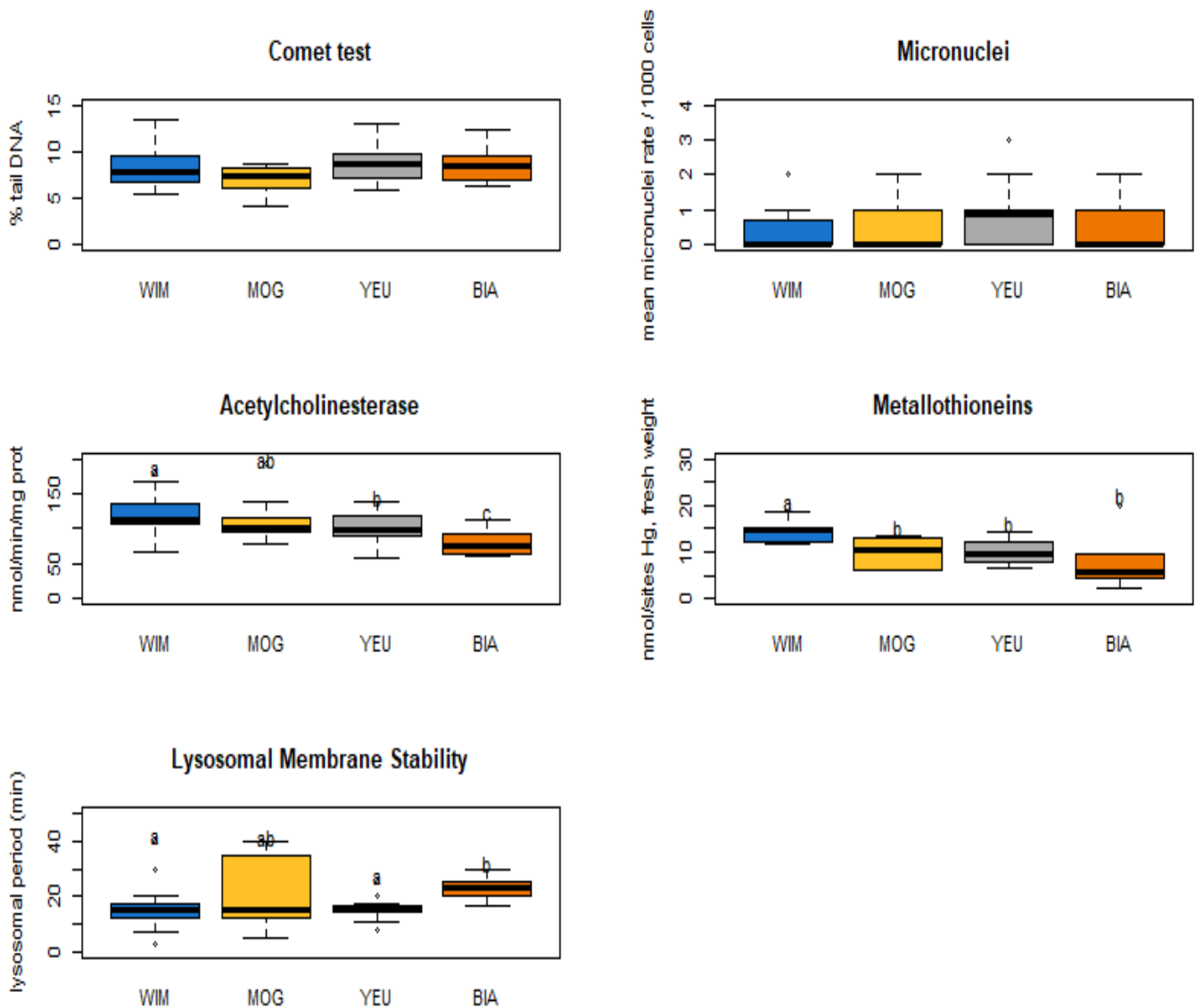
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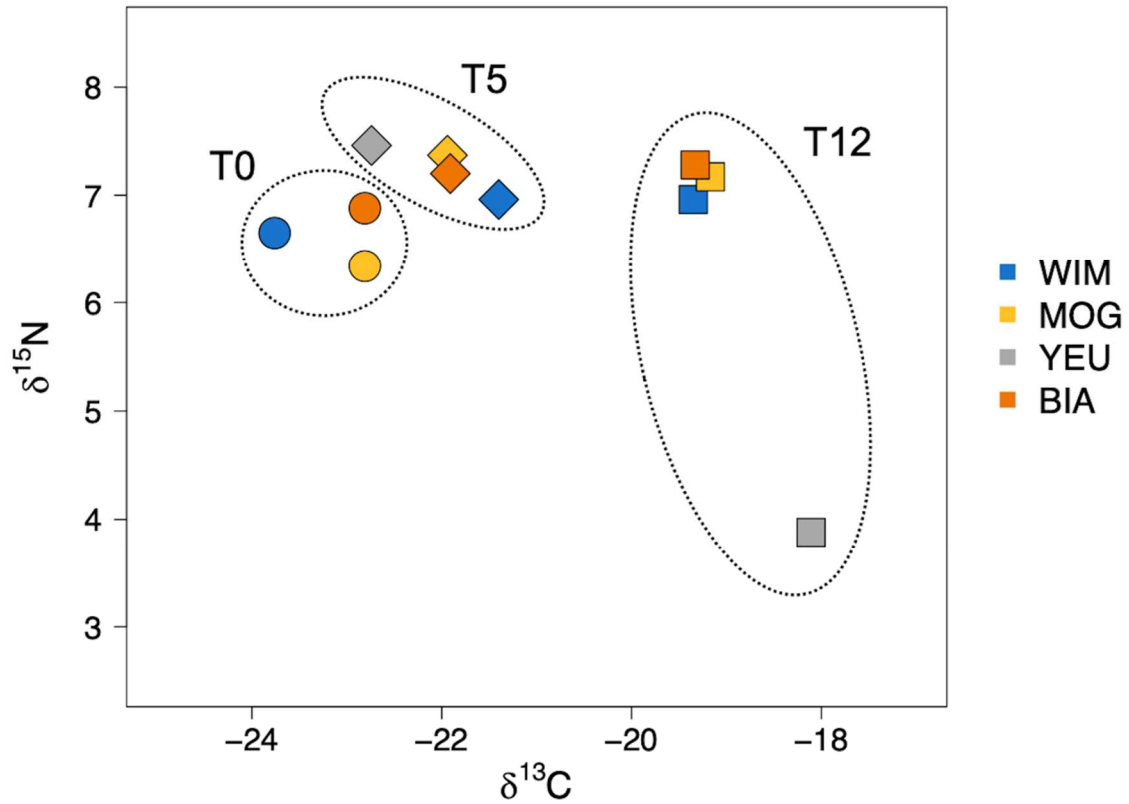
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Figure 2. Distribution of gametogenesis development stages per group (N= 10 individuals) at T12 (0 = quiescent stage, I = start of gonial mitosis, II = meiosis, III = ripe). WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*).



782 Figure 3. Ecotoxicological biomarkers responses at T12: 3a) Level of DNA strand breaks (N=15); 3b) and  
 783 micronuclei (N=15); 3c) Acetylcholinesterase activity (N=15), 3d) Metallothionein content (N=6), 3e)  
 784 Lysosomal membrane stability (N=15). WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly  
 785 Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast:  
 786 mainly Atlantic *M. galloprovincialis*). Letters represent statistically different groups calculated by the Fisher's  
 787 Least Significant Difference post hoc test.



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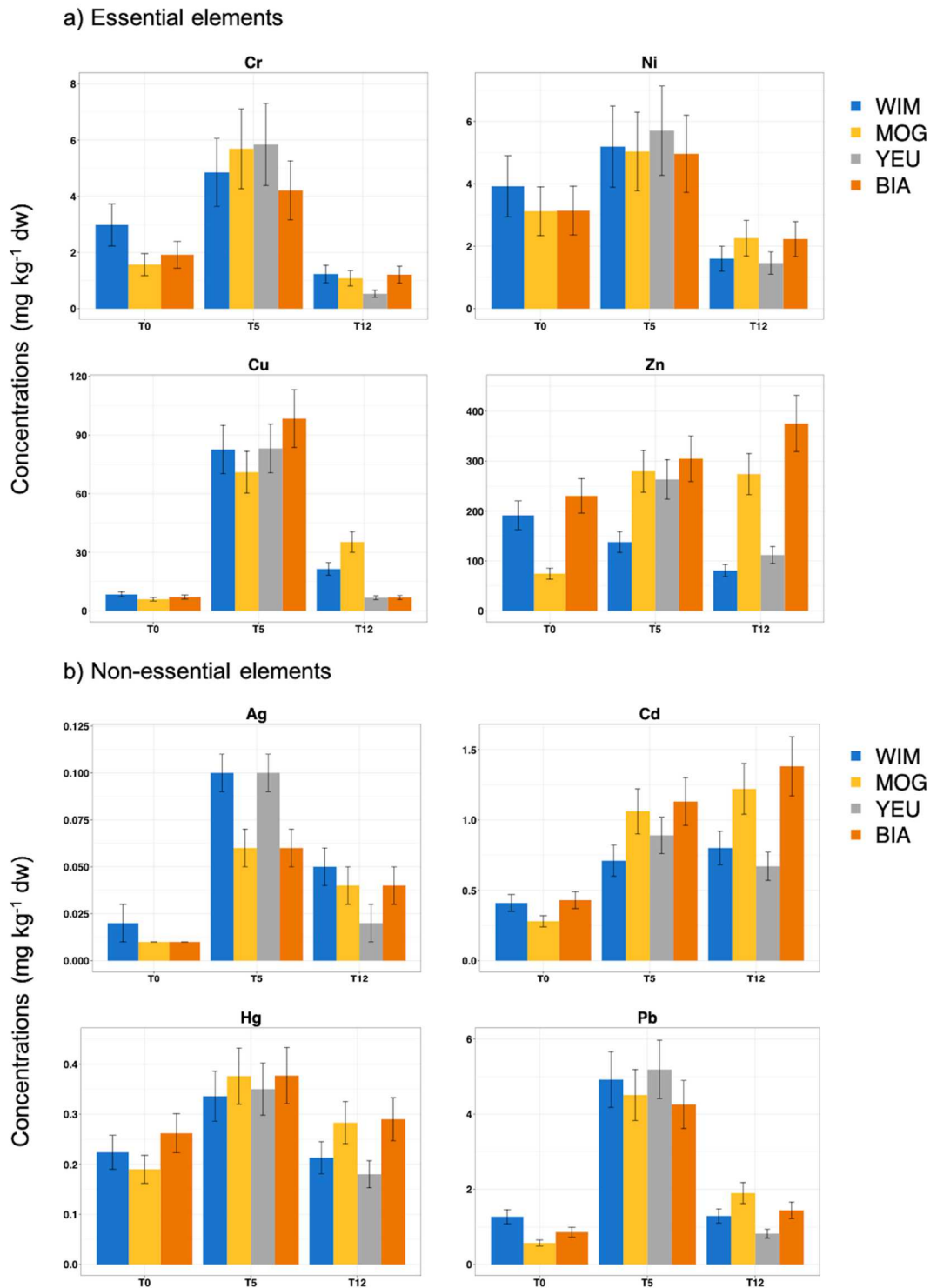
789 Figure 4. Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (in ‰) measured in the mussel progenies over the field experimentation

790 time. Progenies before establishment in the field (T0), 5 months (T5) and 12 months (T12) of establishment.

791 WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed

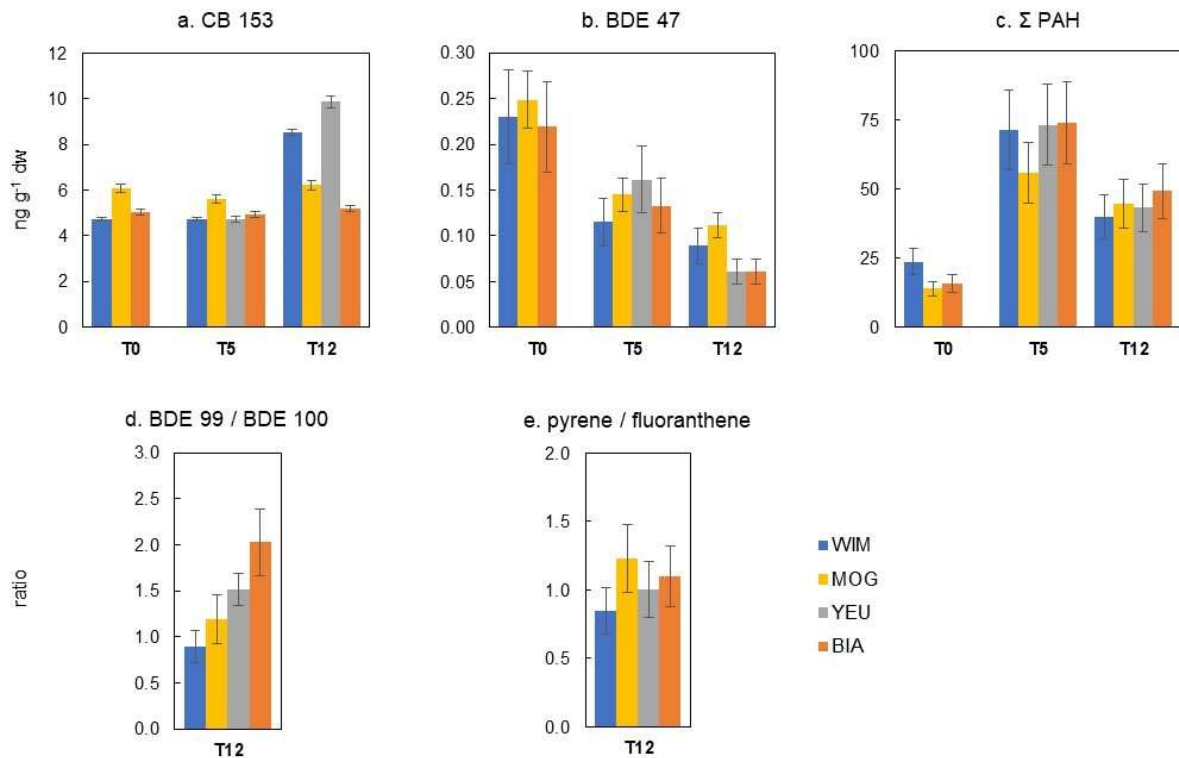
792 with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*.

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794

795 Figure 5. Total concentrations of trace metals measured in the mussel progenies. 5a) Essential elements (Cr,  
 796 Ni, Cu, Zn), 5b) Non-essential elements (Ag, Cd, Hg, Pb). Progenies before establishment in the field (T0), at 5  
 797 months (T5) and at 12 months (T12) of establishment. Error bars correspond to the analytical measurement  
 798 uncertainties estimated for the method used. WIM, Northern France: *M. edulis*; MOG, Northern Brittany:  
 799 mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone, *M. edulis*  
 800 individuals and first-generation hybrids; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*.

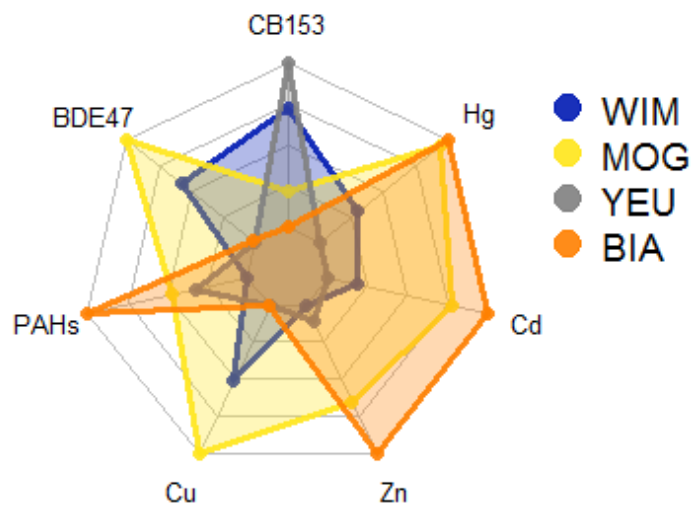


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Figure 6. Organic contaminants measured in the mussel progenies: a) CB 153, b) BDE 47, c) PAH sum (14 congeners), d) BDE 99 to 100 ratio, and e) pyrene to fluoranthene ratio. The error bars represent analytical replicates of the same sample. Groups before field transplantation (T0), 5 months (T5) and 12 months (T12) of transplantation. WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*.

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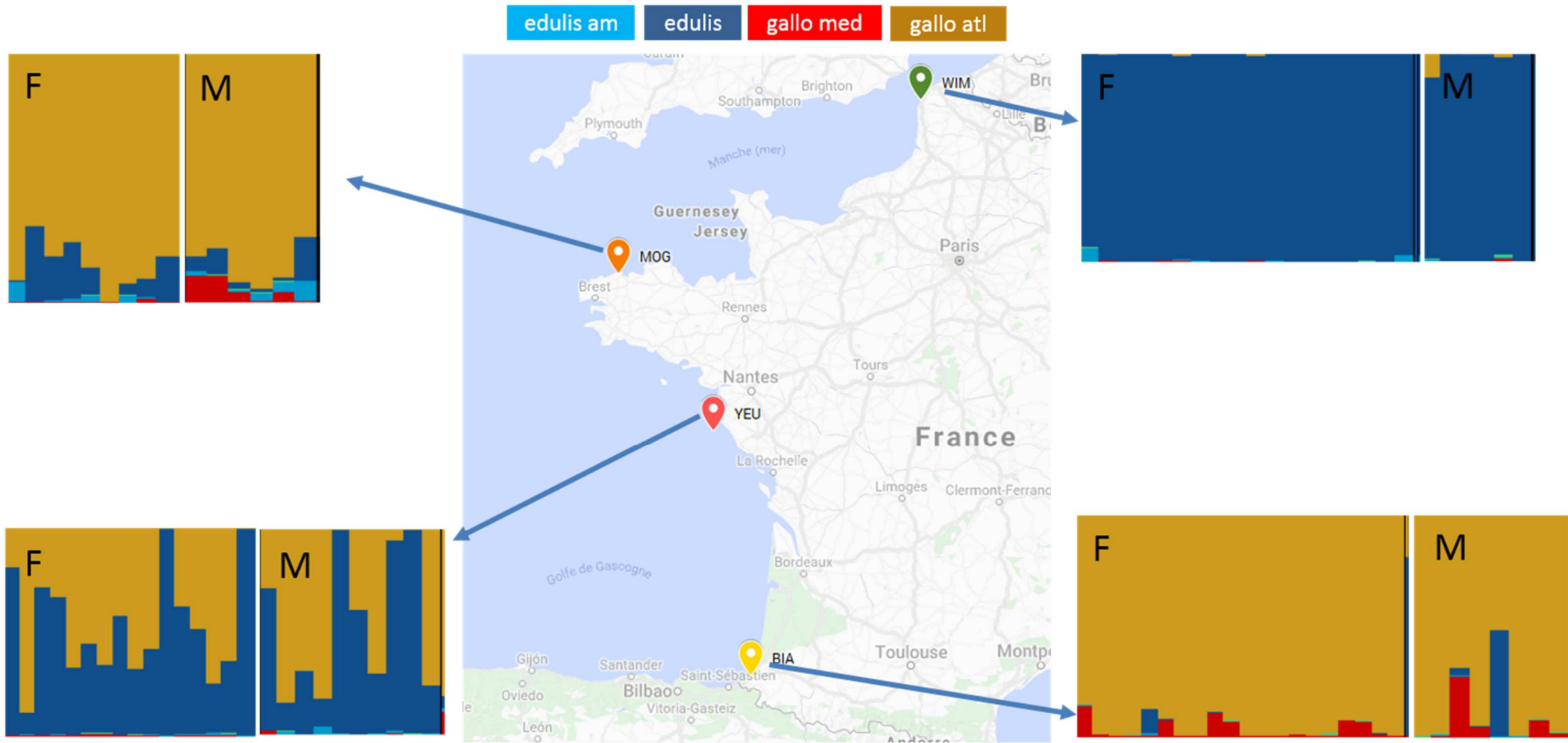
810 Figure 7. Radarplot summarizing the most discriminating contaminants among mussel progenies. WIM,  
 811 Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M.*  
 812 *edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*.

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815 **Appendix A - Origin and genetic structure of the mussel parental populations used in the *in situ* study.**

816 The rectangles indicate the analysis of the genetic structure made with the STRUCTURE software (Pritchard et al., 2000) on the genitors of mussels used in this study (F =  
817 females, M = males). The analyses were carried out on 77 SNPs discriminating between different species and subspecies (Simon et al., 2019). The colors indicate the genetic  
818 background (turquoise blue = *M. edulis* from America; blue = *M. edulis* from Europe; red = *M. galloprovincialis* from the Mediterranean; yellow = *M. galloprovincialis* from  
819 Atlantic). WIM = Wimereux, MOG = Pointe du Moguéric, YEU = Yeu Island, BIA = Biarritz.  
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**Appendix B – Primers used for PCR analysis and genetic results.**

Primers	Sequences
GLU-5' Fw	5'-CCAGTATACAAACCTGTGAAGA-3'
GLU-5' Rv	5'-TGTTGTCTTAATAGGTTTGTAAGA-3'
SNP-001 Fw <i>M. edulis</i>	5'-CGCCTATGCCACCAACGACA-3'
SNP-001 Fw <i>M. galloprovincialis</i>	5'-CGCCTATGCCACCAACGACT-3'
SNP-001 Rv	5'-GGGATAACCCCTGGAATGTT-3'
SNP-064 Fw <i>M. edulis</i>	5'-AAGACATCCGAAAGGCATAT-3'
SNP-064 Fw <i>M. galloprovincialis</i>	5'-AAGACATCCGAAAGGCATAC-3'
SNP-064 Rv	5'-AGTTTTTAAATGATCGTGTGGTAT-3'

823

824 The results for the three genetic markers studied on the 4 groups are given in percentage (N=25). WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic

825 *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*.

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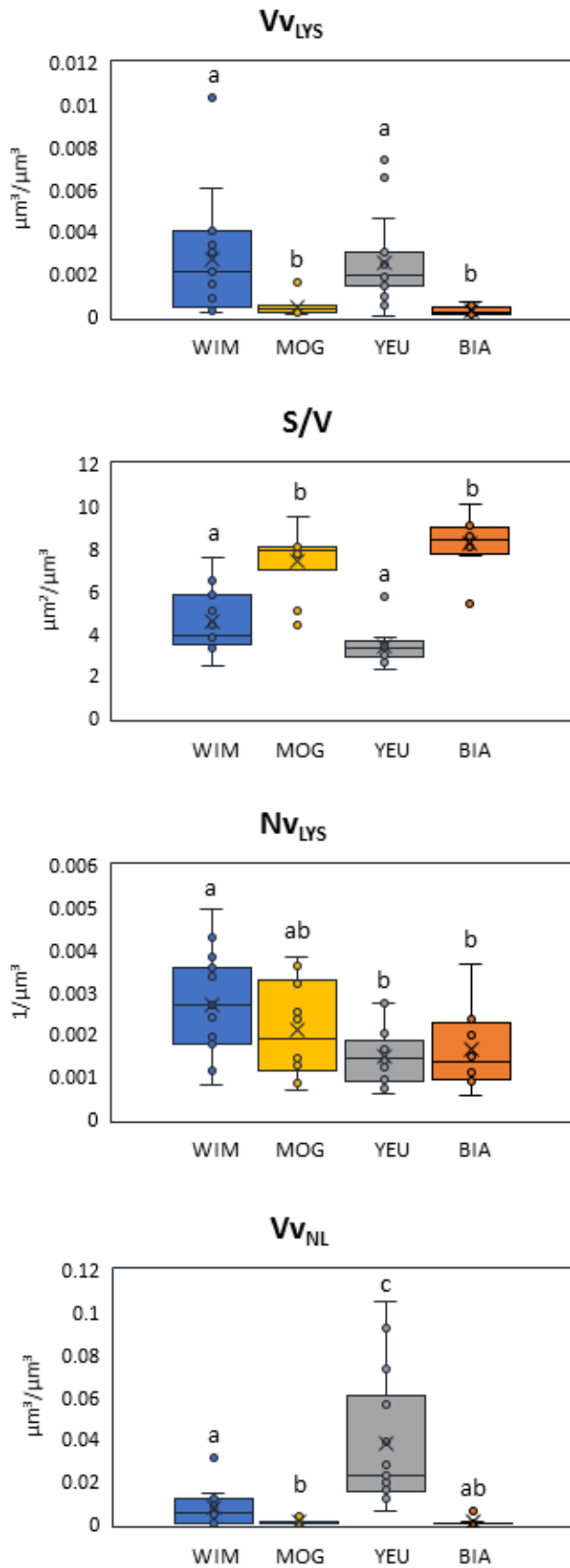
	WIM				BIA				MOG				YEU			
	GLU-5'	SNP-001	SNP-064	average	GLU-5'	SNP-001	SNP-064	average	GLU-5'	SNP-001	SNP-064	average	GLU-5'	SNP-001	SNP-064	average
Homozygote <i>edulis</i>	100	48	84	77	4,2	0	4,4	3	0	4,2	8	4	32,2	20,2	44	32
Homozygote <i>galloprovincialis</i>	0	0	0	0	95,8	83,8	55,4	78	88	52	11,8	51	20	35,8	16	24
Heterozygote	0	52	16	23	0	16,2	40,2	19	12	43,8	80,2	45	47,8	44	40	44

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Appendix C – Lysosomal biomarkers and intracellular neutral lipid accumulation in digestive gland of mussels.  $Vv_{Lys}$ : lysosomal volume density ( $V(L)/V(C)$ ),  $S/V_{Lys}$ : lysosomal surface density ( $S(L)/V(L)$ ),  $Nv_{Lys}$ : lysosomal numerical density ( $N(L)/V(C)$ ) and  $Vv_{NL}$ : volume density of intracellular neutral lipids ( $Vv_{NL}=V(NL)/V(C)$ ); where V=volume, S=surface, N=number, L=lysosomes, NL=neutral lipids and C=cytoplasm.



831 **Appendix D – Values measured for PCBs, BDEs and PAHs in the four mussel progenies.**

832 Progenies before establishment in the field (T0), 5 months (T5) and 12 months (T12) of establishment. WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly

833 Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*. The standard deviation is

834 given by at least 2 replicate measurements on the same sample.

835

836 PCB ng g<sup>-1</sup> dw

	CB 105	CB 114	CB 118	CB123	CB 156	CB 157	CB 167	CB 189	CB 28	CB 52	CB 101	CB 138	CB 153	CB 180	SUM
WIM T0	0.45 ± 0.03	0.01 ± 0.01	1.32 ± 0.06	0.02 ± 0	0.14 ± 0.01	0.05 ± 0.01	0.14 ± 0.01	0.03 ± 0.01	0.09 ± 0.01	0.4 ± 0.01	1.27 ± 0.03	2.22 ± 0.07	4.73 ± 0.12	0.42 ± 0.02	11.3 ± 0.37
BIA T0	0.33 ± 0.02	0.01 ± 0.01	1.07 ± 0.05	0.02 ± 0.01	0.14 ± 0.01	0.06 ± 0.01	0.18 ± 0.01	0.03 ± 0.01	0.08 ± 0.01	0.26 ± 0.01	0.9 ± 0.02	2.28 ± 0.07	5.03 ± 0.13	0.32 ± 0.02	10.7 ± 0.35
MOG T0	0.53 ± 0.03	0.02 ± 0.01	1.7 ± 0.08	0.04 ± 0.01	0.19 ± 0.02	0.06 ± 0.01	0.21 ± 0.01	0.03 ± 0.01	0.1 ± 0.01	0.42 ± 0.02	1.4 ± 0.04	2.66 ± 0.08	6.09 ± 0.15	0.47 ± 0.02	13.9 ± 0.46
WIM T5	0.66 ± 0.04	0.04 ± 0.01	1.76 ± 0.09	0.02 ± 0.01	0.16 ± 0.02	0.06 ± 0.01	0.17 ± 0.01	0.04 ± 0.01	0.09 ± 0.01	0.26 ± 0.01	1.4 ± 0.04	2.86 ± 0.09	4.71 ± 0.12	0.75 ± 0.04	12.98 ± 0.43
BIA T5	0.67 ± 0.04	0.03 ± 0.01	1.82 ± 0.09	0.03 ± 0.01	0.12 ± 0.01	0.03 ± 0.01	0.18 ± 0.01	0.04 ± 0.01	0.12 ± 0.01	0.32 ± 0.01	1.44 ± 0.04	2.66 ± 0.08	4.92 ± 0.12	0.57 ± 0.03	12.95 ± 0.43
MOG T5	0.78 ± 0.05	0.04 ± 0.01	2.11 ± 0.1	0.02 ± 0.01	0.17 ± 0.02	0.07 ± 0.01	0.17 ± 0.01	0.04 ± 0.01	0.1 ± 0.01	0.28 ± 0.01	1.46 ± 0.04	3.13 ± 0.1	5.62 ± 0.14	0.47 ± 0.02	14.47 ± 0.48
YEU T5	0.71 ± 0.04	0.04 ± 0.01	2.05 ± 0.1	0.03 ± 0.01	0.19 ± 0.02	0.07 ± 0.01	0.17 ± 0.01	0.07 ± 0.02	0.13 ± 0.01	0.35 ± 0.01	1.55 ± 0.04	2.8 ± 0.09	4.71 ± 0.12	0.64 ± 0.03	13.5 ± 0.44
WIM T12	0.35 ± 0.02	0.01 ± 0.01	1.22 ± 0.06	0.02 ± 0.01	0.11 ± 0.01	0.04 ± 0.01	0.24 ± 0.01	0.03 ± 0.01	0.09 ± 0.01	0.25 ± 0.01	1.4 ± 0.04	3.74 ± 0.11	8.54 ± 0.22	0.39 ± 0.02	16.42 ± 0.54
BIA T12	0.24 ± 0.01	<0.01	0.8 ± 0.04	0.02 ± 0.01	0.09 ± 0.01	0.03 ± 0.01	0.13 ± 0.01	<0.02	0.11 ± 0.01	0.2 ± 0.01	0.83 ± 0.02	2.36 ± 0.07	5.19 ± 0.13	0.29 ± 0.02	10.31 ± 0.34
MOG T12	0.29 ± 0.02	0.01 ± 0.01	0.96 ± 0.05	0.01 ± 0.01	0.11 ± 0.01	0.04 ± 0.01	0.17 ± 0.01	0.03 ± 0.01	0.15 ± 0.01	0.24 ± 0.01	0.96 ± 0.02	2.76 ± 0.08	6.21 ± 0.16	0.33 ± 0.02	12.27 ± 0.4
YEU T12	0.47 ± 0.03	0.01 ± 0.01	1.7 ± 0.08	0.03 ± 0.01	0.15 ± 0.01	0.05 ± 0.01	0.27 ± 0.01	<0.02	0.11 ± 0.01	0.34 ± 0.01	1.79 ± 0.05	4.32 ± 0.13	9.86 ± 0.25	0.38 ± 0.02	19.51 ± 0.64

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838 PBDE ng g<sup>-1</sup> dw

	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	99/100
WIM T0	0.008 ± 0.003	0.23 ± 0.047	0.098 ± 0.024	0.042 ± 0.006	0.015 ± 0.002	0.011 ± 0.002	ND	2.3 ± 0.4
BIA T0	0.007 ± 0.003	0.219 ± 0.045	0.122 ± 0.03	0.047 ± 0.007	0.011 ± 0.002	0.015 ± 0.003	0.007 ± 0.002	2.6 ± 0.4
MOG T0	0.008 ± 0.003	0.248 ± 0.051	0.087 ± 0.021	0.044 ± 0.007	0.007 ± 0.001	0.01 ± 0.002	0.005 ± 0.001	2 ± 0.3
WIM T5	0.008 ± 0.003	0.115 ± 0.024	0.033 ± 0.008	0.016 ± 0.002	<0.005	0.006 ± 0.001	<0.009	2.1 ± 0.4
BIA T5	0.007 ± 0.003	0.133 ± 0.027	0.053 ± 0.013	0.019 ± 0.003	<0.004	0.01 ± 0.002	<0.006	2.9 ± 0.5
MOG T5	<0.003	0.145 ± 0.03	<0.030	0.026 ± 0.004	<0.038	<0.021	<0.055	
YEU T5	0.014 ± 0.006	0.161 ± 0.033	<0.017	0.03 ± 0.004	<0.028	<0.011	<0.033	
WIM T12	0.002 ± 0.001	0.089 ± 0.018	0.015 ± 0.004	0.017 ± 0.002	0.003 ± 0	0.006 ± 0.001	0.004 ± 0.001	0.9 ± 0.2
BIA T12	0.003 ± 0.001	0.061 ± 0.012	0.03 ± 0.007	0.015 ± 0.002	0.005 ± 0.001	0.007 ± 0.002	0.003 ± 0.001	2 ± 0.4
MOG T12	0.004 ± 0.002	0.111 ± 0.023	0.026 ± 0.006	0.022 ± 0.003	0.003 ± 0	0.006 ± 0.001	0.002 ± 0.001	1.2 ± 0.2
YEU T12	0.002 ± 0.001	0.06 ± 0.012	0.019 ± 0.005	0.012 ± 0.002	0.002 ± 0.001	0.005 ± 0.001	0.002 ± 0.001	1.5 ± 0.3

839

840 PAH ng g<sup>-1</sup> dw

	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benz[a]anthracene	Cyclopenta[cd]pyrene	Chrysene	Benzo[b]fluoranthene	Benzo[j]fluoranthene	Benzo[k]fluoranthene	Benzo(a)pyrene	Indeno[1,2,3-cd]pyrene	Dibenz[a,h]anthracene	Benzo[ghi]perylene	SUM
WIM T0	< 18,6	< 0,50	3.6 ± 0.7	3.9 ± 0.8	1 ± 0.2	0.8 ± 0.2	1.6 ± 0.3	3.4 ± 0.7	1.4 ± 0.3	1.4 ± 0.3	0.8 ± 0.2	2.5 ± 0.5	0.4 ± 0.1	2.4 ± 0.5	23.2 ± 4.6
BIA T0	< 18,6	< 0,50	2.1 ± 0.4	2.3 ± 0.5	0.6 ± 0.1	0.4 ± 0.1	1.1 ± 0.2	2.3 ± 0.5	0.9 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	1.8 ± 0.4	0.2 ± 0	2 ± 0.4	15.3 ± 3.1
MOG T0	< 18,6	< 0,50	2.3 ± 0.5	2.9 ± 0.6	0.4 ± 0.1	0.2 ± 0	0.8 ± 0.2	1.8 ± 0.4	0.7 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	1.4 ± 0.3	0.2 ± 0	1.9 ± 0.4	13.5 ± 2.7
WIM T5	< 21,6	< 1,15	9.3 ± 1.9	9.3 ± 1.9	4.7 ± 0.9	1.8 ± 0.4	7.5 ± 1.5	10.9 ± 2.2	4.6 ± 0.9	4.3 ± 0.9	3.6 ± 0.7	6.1 ± 1.2	1.1 ± 0.2	6.8 ± 1.4	70 ± 14
BIA T5	< 34,2	< 1,82	10 ± 2	9.9 ± 2	5 ± 1	2.1 ± 0.4	7.4 ± 1.5	11.5 ± 2.3	4.6 ± 0.9	4.9 ± 1	4 ± 0.8	5.8 ± 1.2	1.2 ± 0.2	6.2 ± 1.2	72.7 ± 14.5
MOG T5	< 53,8	< 2,86	7.2 ± 1.4	7.6 ± 1.5	3.2 ± 0.6	1.6 ± 0.3	5.5 ± 1.1	9.2 ± 1.8	3.7 ± 0.7	3.9 ± 0.8	2.6 ± 0.5	4.5 ± 0.9	0.9 ± 0.2	5.1 ± 1	54.9 ± 11
YEU T5	< 30,4	< 1,61	9.1 ± 1.8	9.3 ± 1.9	5 ± 1	1.9 ± 0.4	7.8 ± 1.6	11.3 ± 2.3	4.7 ± 0.9	4.9 ± 1	4 ± 0.8	6 ± 1.2	1.2 ± 0.2	6.7 ± 1.3	71.9 ± 14.4
WIM T12	< 18,6	0.6 ± 0.1	7.6 ± 1.5	6.4 ± 1.3	2.7 ± 0.5	0.8 ± 0.2	3.9 ± 0.8	5.8 ± 1.2	2.3 ± 0.5	2.3 ± 0.5	1.4 ± 0.3	2.1 ± 0.4	0.4 ± 0.1	3.5 ± 0.7	39.8 ± 8
BIA T12	< 18,2	1.3 ± 0.3	7.1 ± 1.4	7.8 ± 1.6	3.1 ± 0.6	1.1 ± 0.2	4.4 ± 0.9	7.9 ± 1.6	3.1 ± 0.6	3.8 ± 0.8	2 ± 0.4	3.4 ± 0.7	0.6 ± 0.1	4.1 ± 0.8	49.8 ± 10
MOG T12	< 18,4	0.7 ± 0.1	4.5 ± 0.9	5.6 ± 1.1	2.9 ± 0.6	0.9 ± 0.2	4.2 ± 0.8	8.3 ± 1.7	3.2 ± 0.6	3.7 ± 0.7	1.9 ± 0.4	3.5 ± 0.7	0.6 ± 0.1	4.4 ± 0.9	44.5 ± 8.9
YEU T12	< 18,2	0.7 ± 0.1	6.8 ± 1.4	6.8 ± 1.4	2.9 ± 0.6	0.9 ± 0.2	4 ± 0.8	7.4 ± 1.5	2.7 ± 0.5	2.9 ± 0.6	1.5 ± 0.3	2.3 ± 0.5	0.4 ± 0.1	3.6 ± 0.7	43.1 ± 8.6

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*Mytilus galloprovincialis*

Hybrids?

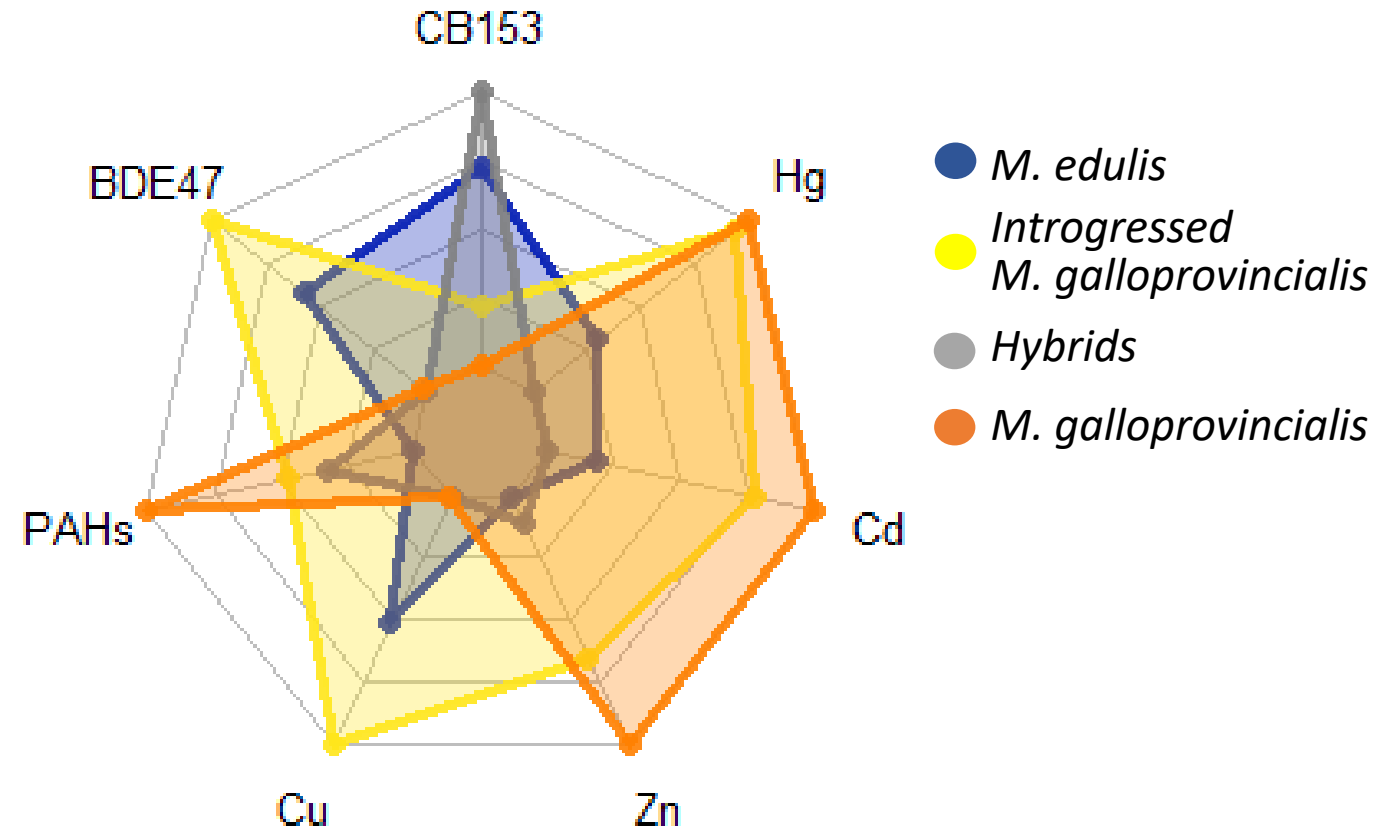


*Mytilus edulis*



In situ transplantation of genetically characterized mussel:

- PCB, PBDE, PAHs, ETM
- Ecotoxicology biomarkers



- Different levels were observed between mussel species for most contaminants and ecotoxicology biomarkers

- Genetics data are an important additional parameter for biomonitoring programs