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

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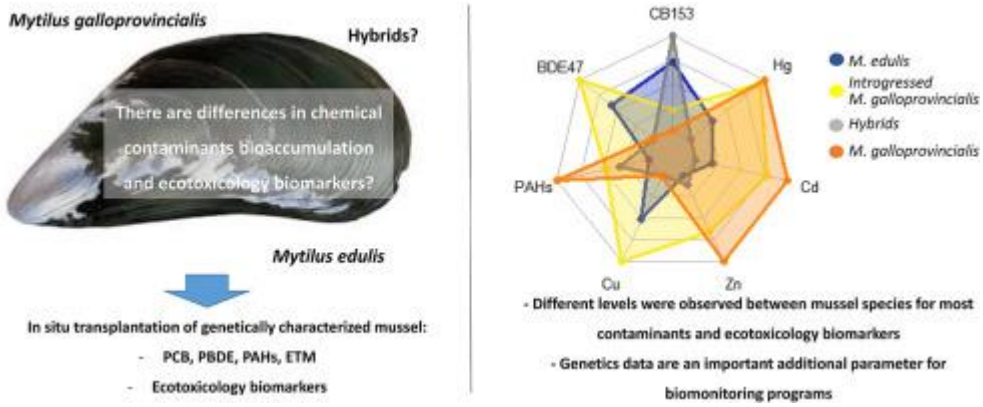
**Abstract :**

The Mytilus mussels are spread all over the world and many related species coexist in several areas and can produce hybrid offspring. Mussels have been used for decades in national and international programs to monitor chemical contamination in the environment. Differences in bioaccumulation and biotransformation abilities between species and their hybrids should be evaluated to assess the comparability of the results obtained within the international biomonitoring programs. The objective of this study was to characterize bioaccumulation abilities and biomarker responses in Mytilus edulis, Mytilus galloprovincialis and their hybrids via an in situ transplantation experimentation on their progenies. Four mussel groups (M. edulis, M. galloprovincialis and two hybrids batches) issued from genetically characterized parents were transplanted for one year in Charente Maritime (France) to ensure their exposure to identical sources of contamination. The bioaccumulation of several families of contaminants (trace metals, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, polychlorinated biphenyls), the response of several biomarkers (DNA strand breaks level, lysosomal membrane stability, metallothionein content, acetylcholine esterase activity) and some physiological parameters (growth, mortality, gonadal development), were analyzed. Differences were observed between species, however they were contaminant-specific. Variations in contaminants levels were observed between progenies, with higher levels of Cu, PBDE, PCB in M. edulis, and higher levels of Cd, Hg, Zn in M galloprovincialis. This study demonstrated that variations in contaminant bioaccumulation and different biomarker responses exist between Mytilus species in the field. Data on species or the presence of hybrid individuals (or introgression) is an important additional parameter to add to biomonitoring programs databases.

## Graphical abstract




## Highlights

► Bioaccumulation and biomarker responses differ between *Mytilus* species in the field. ► Variations in contaminant levels were observed between mussel species. ► Differences were observed between mussel species in biomarkers response. ► Genetics data are an important additional parameter for biomonitoring programs.

**Keywords** : Mussel, Hybridization, Genetics, Metal trace elements, Biomonitoring

## 1. Introduction

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

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

Other studies pinpointed several physiological differences between mussel species such as spawning (Secor et al., 2001), growth (Fuentes et al., 2002; Lobel et al., 1990), parasite infestation (Secor et al., 2001), responses to salinity and temperature (Lockwood et al., 2010; Lockwood and Somero, 2011). The aim of the present study was to assess if bioaccumulation differences and biomarker responses exist between *Mytilus edulis*, *M. galloprovincialis* and their hybrids or introgressed populations, via a one-year *in situ* transplantation experimentation using hatchery-produced mussels whose genetic background is known. The originality of this study consists in comparing genetically characterized mussel species and hybrids responses in the field, allowing to grasp the genetic response. This experimental design also avoid confounding factors such as age (e.g. the older mussel could accumulate higher contaminant concentrations, here all individuals have the same age) and differential acclimation to laboratory or field conditions (here parents having different origins/genetics were maintained in the same conditions since 2016 and all tested individuals were reared in the same location).

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

## 2. Material and Methods

### *2.1 Biological material*

Four groups of mussels were produced from wild populations sampled in four sites along the French coasts, each with a different proportion of *M. edulis* and *M. galloprovincialis* genotypes. These groups were part of the Ifremer MORBLEU project (Pepin et al., 2019). Briefly, wild mussels were collected between October and December 2016 along the French Atlantic coast (Biarritz, BIA, 43.467448 N, -1.576547E; Ile d'Yeu, YEU, 46.70645 N, -2.284001 E; Moguériec, MOG, 48.688292 N, -4.070776E; Wimereux, WIM, 50.786667 N, 1.601944 W, see Appendix A 1 for details), then transported to the Ifremer facilities in La Tremblade and kept in common controlled conditions suitable for germ cell

maturation. All parents were genotyped to determine the species (and level of introgression) to which they belonged as described by Simon et al. (2019). Genotyping was performed with the KASP™ method (Semagn et al., 2014) on 77 nuclear markers in order to differentiate *M. edulis*, *M. galloprovincialis*, hybrids and introgressed individuals. Genetic clustering to determine admixture to either species was performed with the STRUCTURE software (Pritchard et al., 2000), these results are part of those published by Simon et al. (2019). The genetic structure analyses for the studied populations indicated that individuals from Wimereux (WIM, North France) were mainly *M. edulis* (the assignment to *M. edulis* is 99% for females and 88% for males); individuals from Moguériec (MOG, Northern Brittany) had a genetic profile of mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis* (the assignment to *M. edulis* is 19% for females and 11% for males) ; individuals from the Ile d'Yeu (YEU, West coast) presented introgressed *M. galloprovincialis* individuals, *M. edulis* individuals and first generation hybrids the (assignment to *M. edulis* is 52% for females and 52% for males); individuals from Biarritz (BIA, Basque coast) were mainly Atlantic *M. galloprovincialis* (assignment to *M. edulis* is of 0.01% for females and 8% for males) (see Appendix A for details).

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

***2.2 Genetic markers***

In order to confirm the genetic background of the four groups, PCR on the genomic DNA for the GLU-5' (Inoue et al., 1995), SNP-001 and SNP-064 markers (Simon et al., 2019) were performed on the sampled individuals in March 2018 (T5, 10 individuals per group) and in November 2018 (T12, 15 individuals per group, the same individuals were used for the biomarker responses, except for MTs). After the extraction of the genomic DNA from the frozen tissues (EZNA® Mollusc DNA Kit, OMEGA bio-tek), the different PCR were carried out under the following conditions: 200 nM primers (see Appendix B), 200 µM dNTPs, 1.5 mM $MgCl_2$, 1X enzyme buffer (6.70 mM Tris-HCl pH 8.8, 16 mM

($NH_4$) $2SO_4$, 0.01% Tween 20, 2.5 U Taq polymerase (Ozyme)), 200 ng d gDNA. The following programs were used on a Labcycler® thermocycler from Sensoquest: for GLU-5': 95°C-2 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, [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 PCR products, it was possible to discriminate the alleles of the GLU-5' marker for the species *M. edulis* and *M. galloprovincialis* thanks to the size of the amplification products obtained (Inoue et al., 1995). For SNPs, since specific primers to each species were used, the alleles of both species were discriminated by the presence/absence of the amplification product on the gel. Results are expressed as a percentage of individuals with either *edulis* or *galloprovincialis* allele (homozygotes) or both (heterozygous individuals) for each marker.

### *2.3 Physiological parameters*

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

### *2.4 Biomarkers measurement*

At T12, 15 animals per group were sampled for biomarker analyses. All the analyses except metallothioneins were performed on the same individuals. For genotoxicity, the comet assay and the micronuclei test were performed on mussel hemocytes extracted from the adductor muscle. The comet assay was performed as previously described (Revel et al., 2019). Following sampling, individual hemocytes were recovered by centrifugation (1500 rpm, 5 min) of the hemolymph samples. The hemocyte pellet was then resuspended in 160 µL of 0.5% low melting point agarose for the preparation of 2 comet slides. Following lysis and denaturation in the electrophoresis buffer, DNA migration was performed for 15 min at 23 V (390 mA, E = 0.66V $cm^{-1}$). At the end of electrophoresis, the slides were washed by incubation for 3 × 5 min in Tris base 0.4 M, pH 7.5. In order to obtain permanent preparations, the slides were immersed in absolute ethanol for 10 min to dehydrate, then

dried at room temperature. Just prior to analysis, 75 µL of GelRed at 8 mg $L^{-1}$ were spread over each slide using a cover glass. The slides were placed for at least 1 h in the dark at 4°C for coloration, then analyzed using an optical epifluorescence microscope (Olympus BX60, ×40) equipped with a CDD camera (Luca-S, Andor Technology) and image analysis system (Komet 6, Kinetic Imaging Ltd.). At least 50 nuclei were analyzed per slide and the percentage of DNA present in the Comet tail (% Tail DNA) was measured for each observed nucleus. For micronuclei, 100 µL of hemolymph were deposited on a poly-L-Lysine slide. After incubation for 20 min in a humid chamber, the hemocytes were fixed with ethanol/acetic acid solution (3V/1V) for 20 min and then rinsed twice in PBS. The nuclei of the cells were then stained with DAPI (1 $\mu g.mL^{-1}$) for 15 min, then placed between the slide and coverslip with Mowiol mounting liquid. A semi-automated count of micronuclei on 1000 cells per individual was performed on CellInsight CX5 HCS® platform (Thermo).

For acetylcholinesterase activity (AChE), gills were dissected and frozen in liquid nitrogen until analyses. The method described by Ellman et al. (1961) was used, adapted for 96-microplate reader (Bocquéné G., 1998). The activity was measured in S9 fractions from mussel gills. The protein content was determined for each sample according to the Bradford method using BioRad Protein Assay Dye Reagent; bovine serum albumin was used as standard. Each sample was measured in triplicate and absorbance read at 595 nm. One hundred µg of protein (S9 fraction at 1 $\mu g.\mu L^{-1}$) were added to 250 µL of 2 mM sodium phosphate buffer (pH 7, containing 0.1% of TritonX-10); then 20 µL of dithio*bis*nitrobenzoate 10 mM (Sigma – Ref D8130) and 10 µL of acetylthiocholine 100 mM (Sigma – Ref D5751) were added. AChE activity was measured by reading the optical density on a microplate reader (Safire TECAN) at 412 nm after 2 min incubation at room temperature under gentle shaking. Results are given as $units.min^{-1}.mg\ protein^{-1}$.

For lysosomal biomarkers, digestive glands were dissected out and frozen in liquid nitrogen until analyses. The lysosomal membrane stability (LMS) was determined in serial cryotome sections (10 µm) of mussel digestive gland after demonstration of hexosaminidase (Hex) activity, according to UNEP/RAMOGE (1999). The time of acid labilisation treatment required to produce the maximum staining intensity was assessed under a light microscope and was denoted as the Labilisation Period (LP; in min). Four determinations were made per individual; for each area, the first maximum staining peak was considered to determine the LP value (Martínez-Gómez et al., 2015). A final LP value was calculated for each individual as the mean of the four LP values determined in each area. For Lysosomal structural changes (LSC), the visualisation of lysosomes in digestive cells of *Mytilus* sp. was based on the histochemical demonstration of β-glucuronidase activity, according to the procedure described by Moore (1976) with the modifications made by Cajaraville et al. (1989). The structure of lysosomes was assessed through a stereological procedure based on image analysis (BMS, Sevisan) according to Cajaraville et al. (1991). Five measurements using a 100x objective lens were made per

individual. The mean value of the following stereological parameters was determined for each mussel digestive gland (Lowe et al., 1981): lysosomal volume density ($Vv_{LYS}$); lysosomal surface to volume ratio ($S/V_{LYS}$) and lysosomal numerical density ($Nv_{LYS}$). Intracellular neutral lipids were histochemically demonstrated in crytome sections (8 µm) of mussel digestive glands stained with Oil Red O (ORO) (Culling 1974). Neutral lipid volume density ($Vv_{NL}$) was determined through a stereological procedure using image analysis (BMS, Sevisan) by measurement of five areas per individual, using a 40x objective lens.

Metallothioneins (MTs) concentrations were analyzed on the full animal flesh (6 replicates per group) with the indirect method of inorganic mercury saturation as described by Baudrimont et al. (2003). For this measure, flesh was immediately placed in liquid nitrogen until measurement. The device used to measure the mercury is an atomic absorption spectrometer by heat treatment under oxygen flow (AMA 254, SYMALAB), whose limit of detection is 1 ng of mercury.

### *2.5 Chemical analyses*

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

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

Aliquots of homogenised powder (0.40 ± 0.05 mg) were weighed in tin cups. Analyses were performed with a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Thermo Scientific Flash EA1112 elemental analyser. The results are presented in the usual $\delta$ notation relative to the deviation from international standards (Pee Dee Belemnite for $\delta^{13}C$ values, and atmospheric nitrogen for $\delta^{15}N$ values), in parts per thousand (‰). Based on replicate measurements of internal laboratory standards (acetanilide), the experimental precision was <0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$. Variations in organisms' lipid content may affect $\delta^{13}C$ values, while food sources do not change. This is because lipids are considerably depleted in $^{13}C$ compared to other tissue components (DeNiro and Epstein, 1977). Moreover, lipid content can vary greatly according to the season in bivalves, as shown by seasonal variations in their C/N ratio (i.e. proxy of lipid content; Briant et al., 2018). In order to compare isotopic compositions of bivalves over the study period, $\delta^{13}C$ values were thus corrected according to the formulae of (Post et al., 2007).

### *2.5.2 Trace metal analyses*

Total mercury (Hg) in mussels was assessed by atomic absorption spectrophotometry on aliquots of homogenised powder (50 ± 5 mg), using an Advanced Mercury Analyser (ALTEC AMA-254, Altec Ltd) and according to the standard operating procedure described in the US-EPA method N°7473 (U.S. Environmental Protection Agency, 1998). The limit of quantification (LOQ) was 0.015 mg $kg^{-1}$ dry weight (dw). Total chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), silver (Ag), cadmium (Cd) and lead (Pb) concentrations were determined with a Quadrupole Inductively Coupled Plasma Mass Spectrometer (Q-ICP-MS, ICAP-Qc model from ThermoFisher), according to an in-laboratory approved method. Briefly, aliquots of samples (~200 mg of homogenised powder) were placed in Teflon bombs and mineralized with a mixture of ultrapure $HNO_3$ acid and ultra-pure water using a microwave digestion system (ETHOS-UP model from Milestone). The digests were then diluted to 50 mL with ultra-pure water before analyses with Q-ICP-MS. With this method, LOQs (in mg $kg^{-1}$ 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 quality assurance of all metal analyses relied on blank and internal standard controls, and on the accuracy and reproducibility of data relatively to the certified reference materials (CRMs) used for analytical runs. Blank values were systematically below the detection limits and CRM values concurred with certified concentrations, with recovery rates ranging between 85% and 101%. The CRMs used were IAEA-142 (mussel homogenate; International Atomic Energy Agency) for Hg, and CE-278k (mussel tissue, Joint Research Centre- European Commission) and SRM-1566b (oyster tissue, National Institute of Standards and Technology) for the other trace metals.

### *2.5.2 Organic contaminants measurement*

For PCB, PBDE, and lipid content, ground freeze-dried tissues (1 g) were extracted by pressurized liquid extraction (PLE - SpeedExtractor - Buchi, France) with a mixture of toluene/acetone (70/30). The extracts were sequentially cleaned-up on acidified silica and florisil. PCB (dioxin-like: 105, 114, 118, 123, 156, 157, 167, 189; and non-dioxin-like: 28, 52, 101, 138, 153, 180) and PBDE (28, 47, 99, 100, 153, 154, 183) congeners were screened in the final extract by Gas Chromatography – High Resolution Mass Spectrometry (GC-HRMS – magnetic sector). Quantification was performed by isotopic dilution using the $^{13}C$-labelled homologue of each of the analytes. For PAH analysis, freeze-dried tissues (0.3 g) were extracted by a mixture of hexane/acetone (50/50) in a PLE system and the extracts were further purified on a styrene-divinylbenzene copolymer sorbent. Analysis was performed on a gas chromatograph coupled with a tandem quadrupole mass spectrometer (GC-MS/MS) and PAHs were quantified against their D- or $^{13}C$-labelled homologue. For all classes of organic contaminant and in each set of 10 samples, a blank sample and a quality control were also analyzed. At least three technical replicates were performed for all analyses.

### *2.6 Statistics*

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

## 3 Results

### *3.1 Genetic and physiological parameters*

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

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

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

### *3.2 Ecotoxicological biomarkers*

Average DNA strand breaks were 8.3 ± 2.1% (min = 4.1%, max = 13.5%), no significant differences were found between groups (Figure 3a). The micronuclei test showed levels comprised between 0‰ and 3‰, and did not show any significant differences between the four mussel groups (Figure 3b). Acetylcholinesterase activity (AChE) showed overall mean levels of 103 nmol/min/mg prot and

differences between groups (*p*-value = 0.015) with lower levels for BIA group (Figure 3c). Metallothionein capacity showed higher levels for the WIM group (*p*-value = 0.032, Figure 3d). In lysosome membrane stability (LMS) test, the mean lysosomal period (LP) was 19.0 ± 1.2 min. The highest LP values were recorded in *M. galloprovincialis* (BIA: 22.9 ± 1.2 min and MOG: 21.6 ± 3.7 min) and the lowest values were recorded in *M. edulis* (WIM 16.0 ± 2.3 min) and in hybrids mussels from YEU (15.5 ± 1.0 min) (*p*-value = 0.002, Figure 3e).

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

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

### *3.3 Isotope analyses*

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

### *3.4 Trace metal analyses*

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

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

### *3.5 Organic contaminants*

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

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

## *4* Discussion

### *Physiological parameters*

The aim of this study was to investigate whether mussel genetics could affect physiological parameters, ecotoxicological biomarker and contaminant bioaccumulation. Progenies of 4 mussel populations presenting different genetic backgrounds, produced and grown under the same conditions were transplanted *in situ* and showed significant differences on physiological traits such as growth and mortality. As already observed in a laboratory experiment following copper exposure, Meat yield was lower for *M. galloprovincialis* compared to *M. edulis* (Brooks et al., 2015). Moreover here, the highest Meat yield was observed for the YEU group (mussel progeny from the Ile d'Yeu) originating from a hybrid zone including individuals from both species, introgressed and hybrids individuals. Given the presence of hybrid individuals in this group, these results could be linked to the concept of heterosis (hybrid vigour), already demonstrated in *M. edulis*/*M. galloprovincialis* hybrids (Bierne et al., 2002), hybrids having the ability to faster adapt to a large range of new

conditions. Differences in Meat yield or condition index are related to different growth rates between species. Indeed age to size correlations vary not only from geographical areas but also depending on the studied species (Izagirre et al., 2014). Age differences for individuals of the same size were related to differences in contaminant bioaccumulation between *M. edulis* and *M. trossulus* (Lobel et al., 1990), and might induce bias in biomonitoring observations (i.e. older mussel presenting higher contaminant levels). To solve this bias, (Izagirre et al., 2014) recommended mussel age standardization in mussel biomonitoring program. Concerning mortality, in the present study *M. galloprovincialis* mussels (BIA *M. galloprovincialis* progeny from Basque Coast and MOG *M. galloprovincialis* progeny from Brittany) had higher mortality rates than the other species. These same populations were tested on several other sites in the framework of the Ifremer MORBLEU projects (Pepin et al., 2019), and significant population mortality was found by site interaction. MORBLEU data showed that during episodes of abnormal mortality outbreaks, *M. edulis* was more sensitive than *M. galloprovincialis*, their hybrids being intermediates (Pepin et al., 2019). However, in the absence of abnormal mortalities, *M. galloprovincialis* seems less adapted to the Marennes-Oléron Bay conditions compared to *M. edulis*, which is linked with the distribution of *Mytilus* species along the French Atlantic coast, with *M. galloprovincialis* North and South of the Marennes-Oléron Bay and Charentais. In our study, sound differences on gametogenesis stages seemed to subsist among the four mussel populations. In the annual cycle of *M. edulis,* gonads start to redevelop in November to become fully mature in early spring (Seed, 1975). Furthermore, the gametogenesis cycles of the two species seem to vary within the same site (Bayne, 1976), and an *in situ* study showed that populations of *M. edulis* and *M. galloprovincialis* coexisting in southern England had a gametogenesis and egg-laying lag of around eight weeks, *M. galloprovincialis* reproducing later than *M. edulis* (Seed, 1971). This seems consistent with our results, the WIM group (*M. edulis* progeny from North of France) being in gametogenesis stages I or II, while BIA being mainly at stage 0 and I. Here the YEU progeny showed a greater heterogeneity in gametogenesis stages, which might reflect its genetic heterogeneity. Moreover, YEU had more individuals at gametogenesis stage II, potentially linked with the higher Meat yield observed, or a better adaptability to the local conditions of the transplantation site. These results also concern only 10 individuals per progeny, which represents a relatively small number in order to be able to draw general conclusions on the different genotypes. In a biomonitoring perspective and according to CEMP/OSPAR (2018), biomonitoring should be performed out of the active spawning season (spawning peaks are in springtime and autumn, when individuals may lose up to 50% of their soft tissue weight). Here if the differences in Meat yield observed stay comparable between groups in November outside of the active spawning season, differences in the gametogenesis stages are found between *Mytilus* species, potentially leading to bias in flesh lipid contents directly influencing contaminant affinity to tissues.

***Ecotoxicology biomarkers***

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

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

Even if performed only on a limited panel of nuclear markers, here the correlation tests results confirms that individuals with more *M. edulis* alleles seems more sensitive to general stress (higher values for % Tail DNA in the Comet test and lower levels of LMS) but have better anti-neurotoxicity

capabilities (higher AChE levels). Anyway, to generalize such results to a specie or genetic background more investigation are need using more populations and more locations.

***Chemical analysis***

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

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

Trace metal regulation mechanisms also seem to be different in the four progenies, as WIM batch presents at T12 the highest metallothionein (MTs) levels and is also the one that has lower Zn, Cd and Hg concentrations than BIA and MOG, but higher Cu concentrations compared to BIA (Figure 7). Interestingly, other studies found higher MT concentrations in *M. galloprovincialis* compared to *M. edulis*, and in the first species MTs induction seemed to depend on several metals, while in *M. edulis* essentially on Cd (Amiard et al., 2006; Zorita et al., 2007). In marine organisms and especially in bivalves, MTs are involved in the homeostasis of essential trace elements such as Cu and Zn, but also in the detoxification of certain non-essential metal trace elements such as Cd, Ag or Hg. MTs complex these ions and store them in an inert form, or even allow their elimination (Amiard et al., 2006). Thus, higher MTs activity/concentration would be induced during a significant exposure of

organisms to trace metals, but it may also be induced by other environmental stresses such as an increase in temperature (Le et al., 2016; Wang and Rainbow, 2010). On the other hand, MTs activity might also be constitutionally higher in some species, explaining lower levels of metal trace elements (Amiard et al., 2006). Therefore, the processes involved in these mechanisms are still poorly understood, and the significance of high levels of MTs measured in organisms is still subject of debate.

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

PCB concentrations varied between progenies, with higher values in YEU and WIM (hybrid zone and *M. edulis*), compared to BIA and MOG (mostly *M. galloprovincialis*). These trends were observed in both dry weight and lipid weight normalized concentrations, suggesting they did not relate to the tissue's biochemical composition. They could eventually be attributed to (i) a higher filtration rate, and therefore a higher exposure to environment contamination or (ii) a more effective elimination process in other groups. Furthermore, YEU progeny was the one with highest Meat yield, to be potentially linked with higher filtration rates. Unfortunately, data on differential filtration rates and metabolization processes between these two species are lacking to fully explain these results. As for metal trace elements, lower BDEs concentrations are observed for T5 and T12 compared to T0, indicating biotransformation and excretion and / or biodilution by growth. Interestingly, since biota exposure pathways are similar for PBDEs and PCBs, this BDE-specific decrease seems to indicate elimination rather than biodilution which would also have affected PCBs. In our study BDE concentrations were higher in MOG and WIM progenies, making difficult to draw any conclusion on potential genetic effects. Studies on fish showed that the penta-BDE congeners 99 and 100 do not have the same susceptibility to biotransformation since congener 99 is metabolized by debromination when BDE 100 is not, despite their similar physico-chemical properties (Munschy et al., 2011). First order kinetics of relatively comparable constants were found in mussels for both PCBs and PBDEs in lab conditions (Gustafsson et al., 1999). However the strength of our study is to be

under real environmental conditions in the marine environment. Unlike lab-based experiments, phytoplankton and particle contaminations are not characterized and are expected to vary significantly. As such, interpretation of results against uptake/metabolisation/depuration kinetics requires caution. If this approach remains valid for bivalves, the PBDE 99/100 congener *ratio* (6b) can therefore be an indicator of metabolic capacities, where high values would indicate low metabolic capacities. At T12, this ratio is less important for WIM suggesting higher metabolic capacities for *M. edulis*. Using a similar approach, no difference was found between progenies on PCB congeners of different susceptibility to metabolization in fish and mammals (Boon et al., 1997; Buckman et al., 2006). Concerning PAHs, concentrations increased at T5 and lowered at T12 indicating different accumulation / metabolization over time as for BDE. If at T12 no variations could be observed for the sum of PAHs between the four progenies, pyrene/fluorantene ratio, also indicating metabolization capacities (Arnot et al., 2008), showed lower levels for WIM, also suggesting a better metabolization capacity for PAHs in this progeny as already suggested for BDEs.

**Conclusion**

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

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

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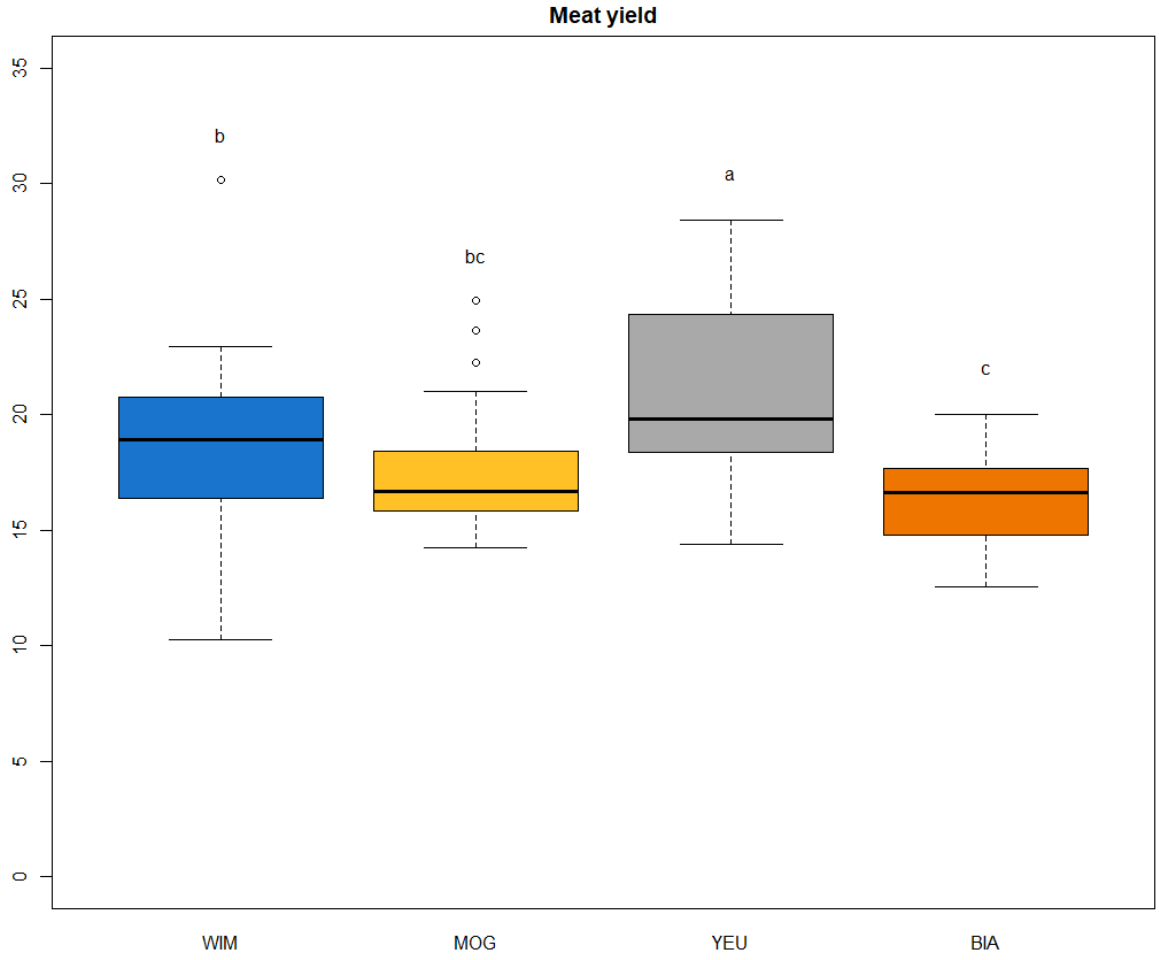



Figure 1. Meat yield of the four mussel progenies at the end of the field experimentation (T12) (WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis*).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

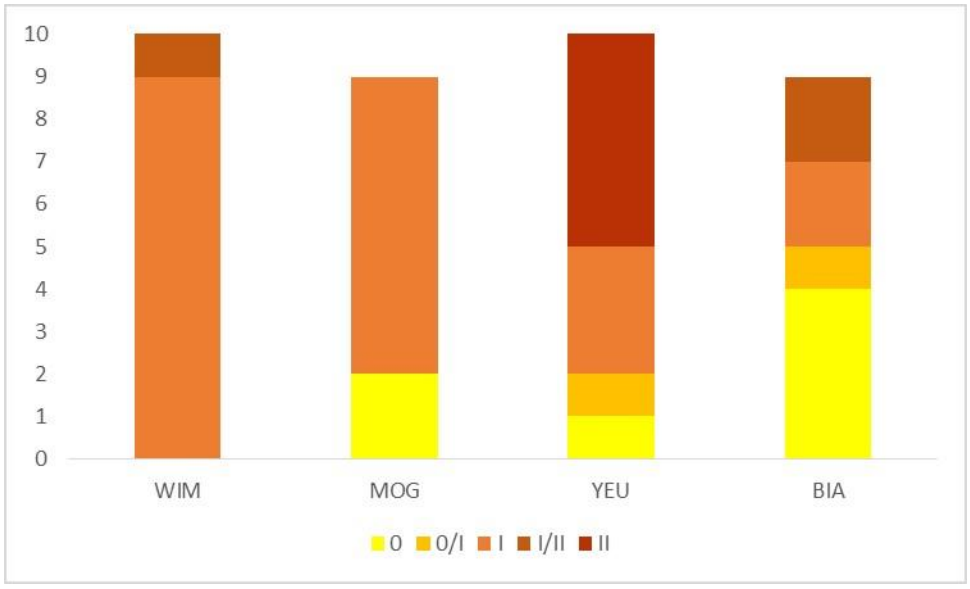


Figure 2. Distribution of gametogenesis development stages per group (N= 10 individuals) at T12 (0 = quiescent stage, I = start of gonial mitosis, II = meiosis, III = ripe). WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*;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

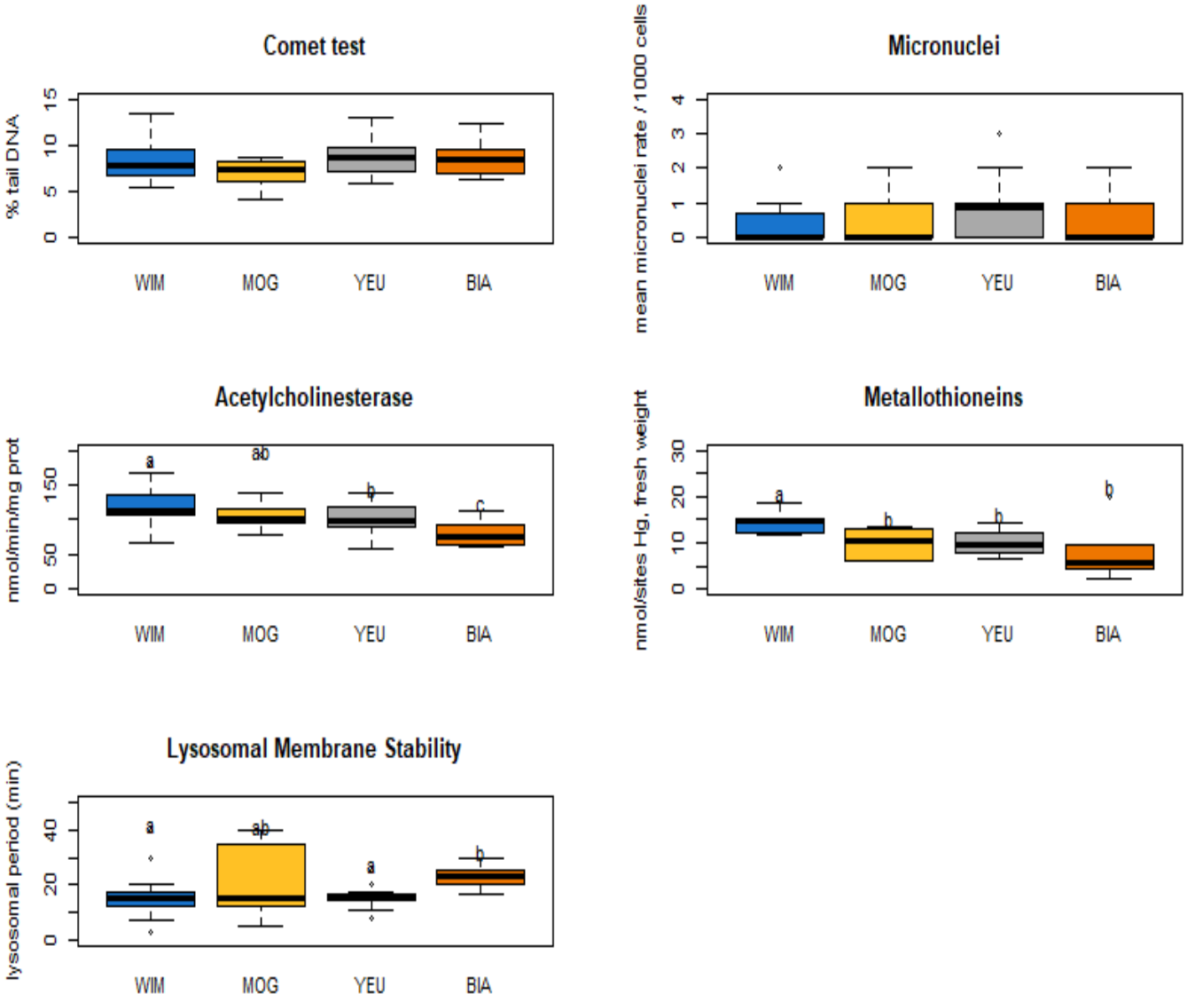



Figure 3. Ecotoxicological biomarkers responses at T12: 3a) Level of DNA strand breaks (N=15); 3b) and micronuclei (N=15); 3c) Acetylcholinesterase activity (N=15), 3d) Metallothionein content (N=6), 3e) Lysosomal membrane stability (N=15). WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly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

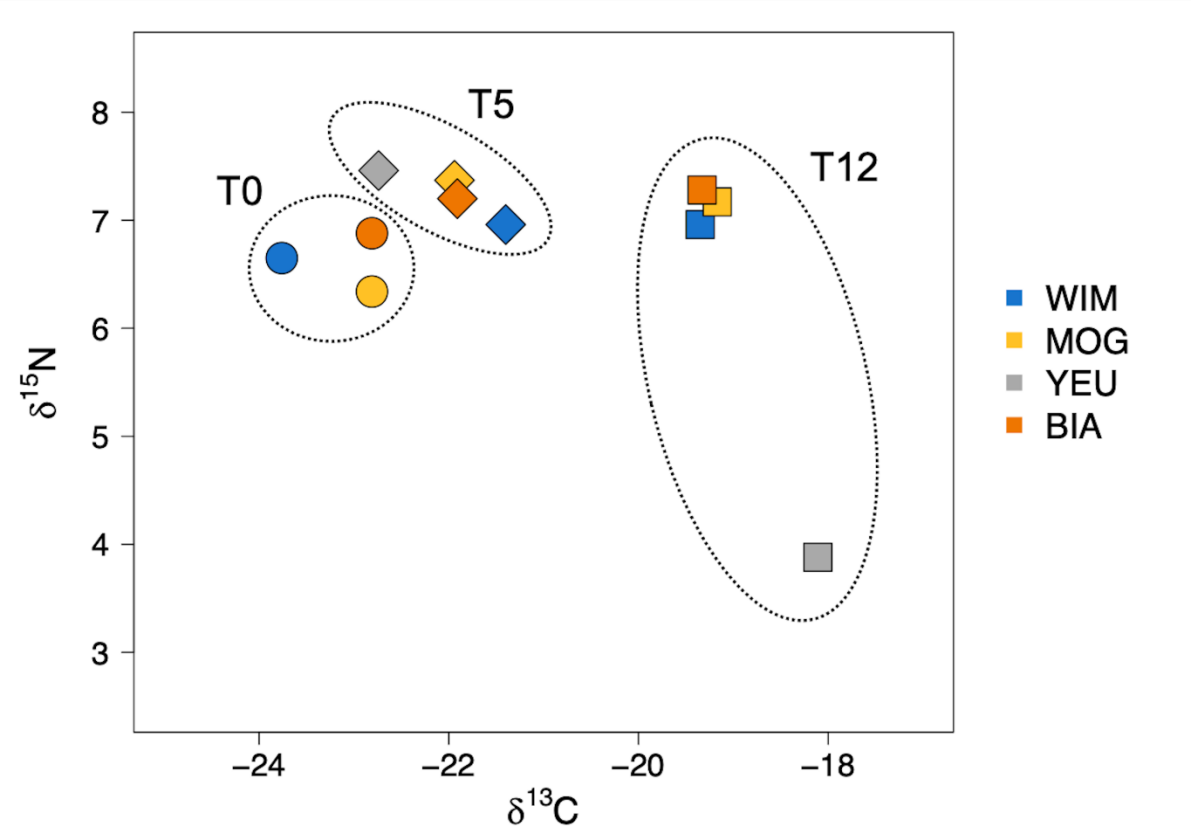



Figure 4. Values of $\delta^{13}C$ and $\delta^{15}N$ (in ‰) measured in the mussel progenies over the field experimentation time. Progenies before establishment in the field (T0), 5 months (T5) and 12 months (T12) of establishment. WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis.*

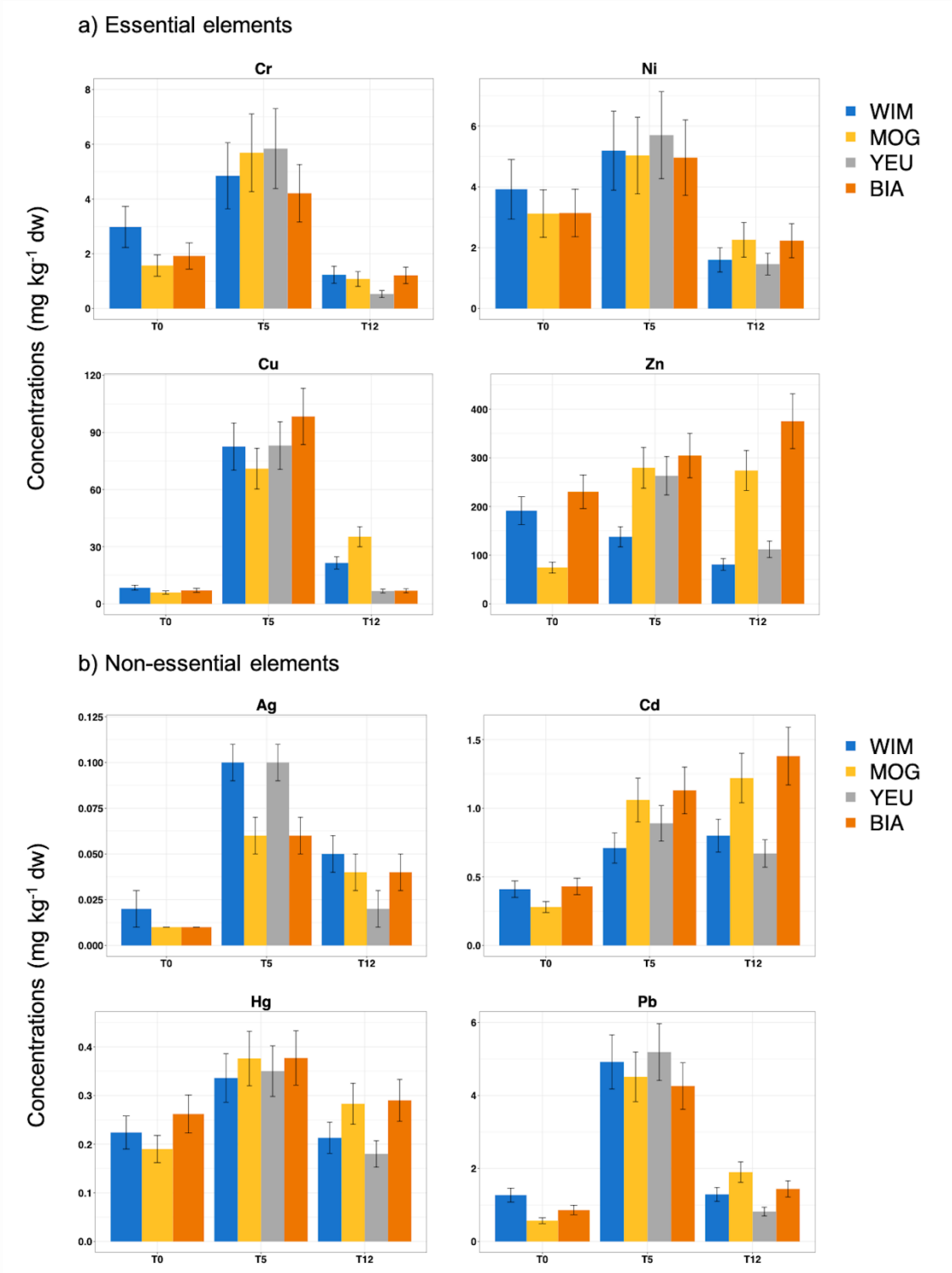



Figure 5. Total concentrations of trace metals measured in the mussel progenies. 5a) Essential elements (Cr, Ni, Cu, Zn), 5b) Non-essential elements (Ag, Cd, Hg, Pb). Progenies before establishment in the field (T0), at 5 months (T5) and at 12 months (T12) of establishment. Error bars correspond to the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

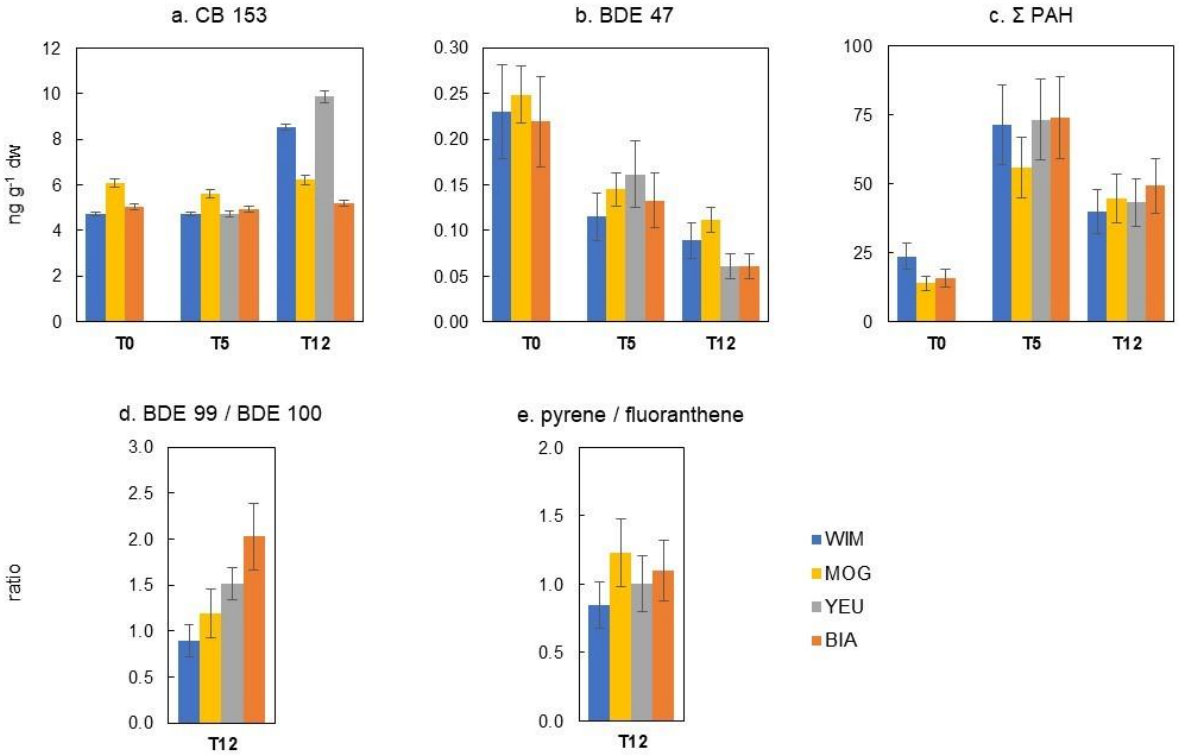



Figure 6. Organic contaminants measured in the mussel progenies: a) CB 153, b) BDE 47, c) PAH sum (14 congeners), d) BDE 99 to 100 ratio, and e) pyrene to fluoranthene ratio. The error bars represent analytical replicates of the same sample. Groups before field transplantation (T0), 5 months (T5) and 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.*

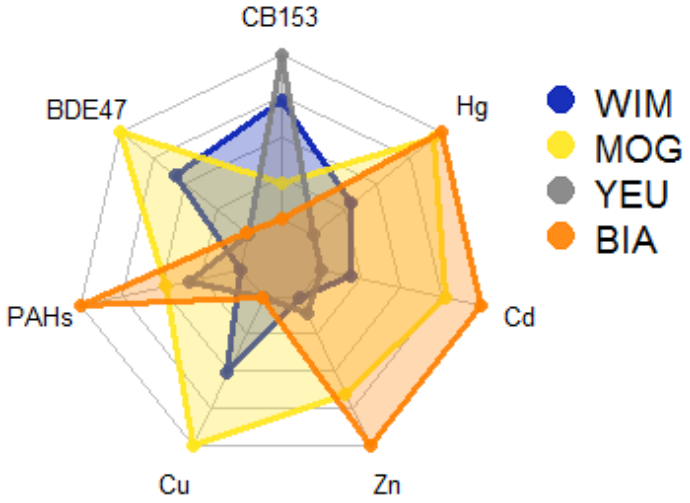
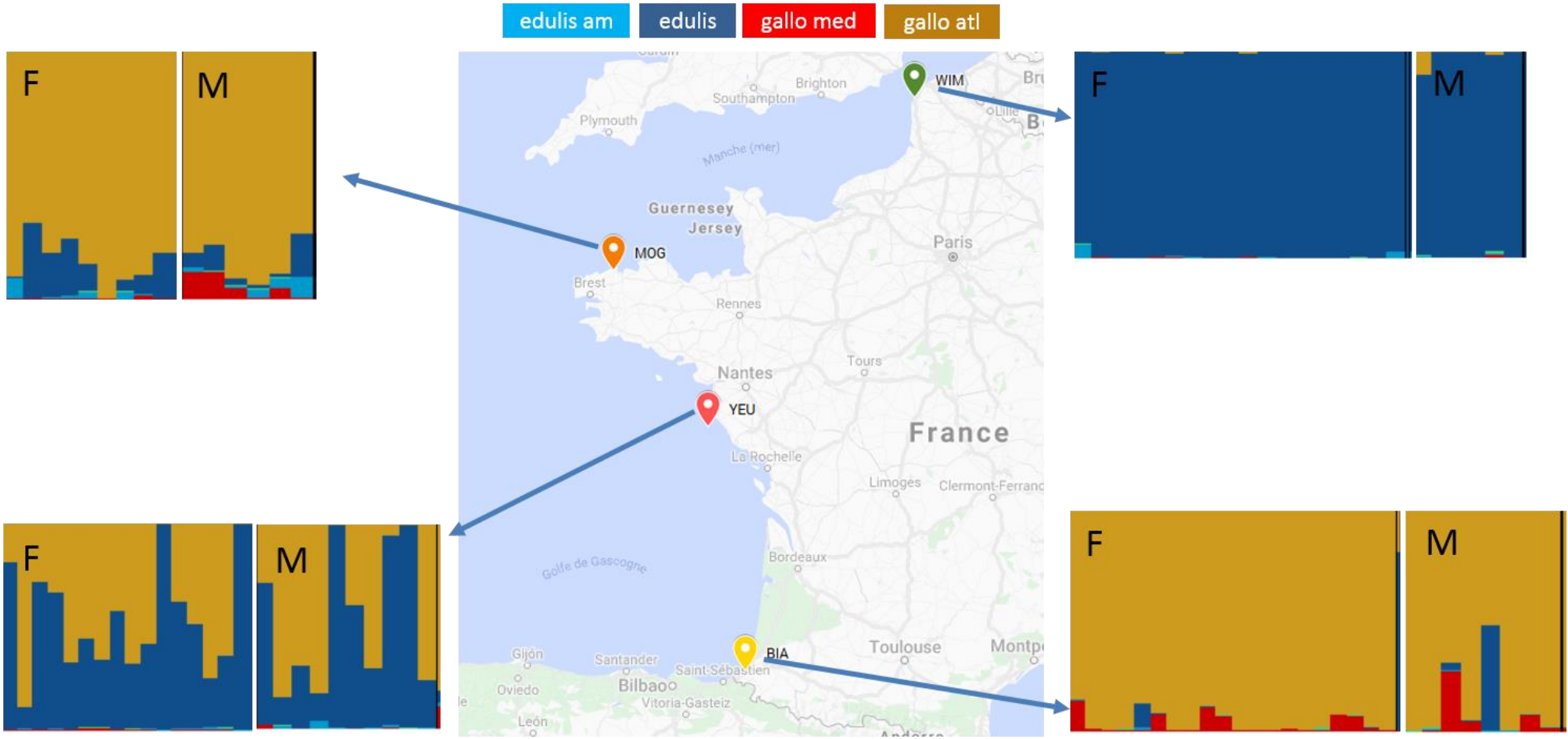



Figure 7. Radarplot summarizing the most discriminating contaminants among mussel progenies. WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis.*

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

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 = 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 background (turquoise blue = *M. edulis* from America; blue = *M. edulis* from Europe; red = *M. galloprovincialis* from the Mediterranean; yellow = *M. galloprovincialis* from Atlantic). WIM = Wimereux, MOG = Pointe du Moguériec, YEU = Yeu Island, BIA = Biarritz.



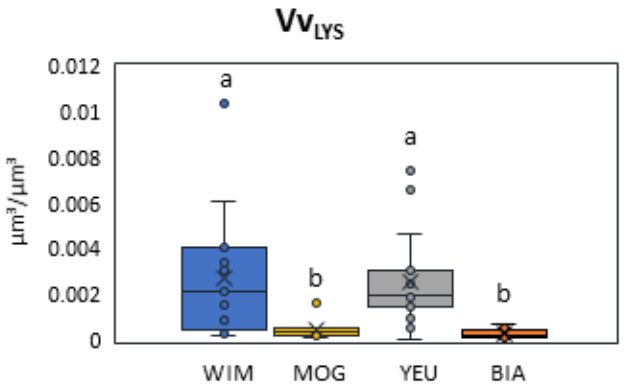
**Appendix B – Primers used for PCR analysis and genetic results.**

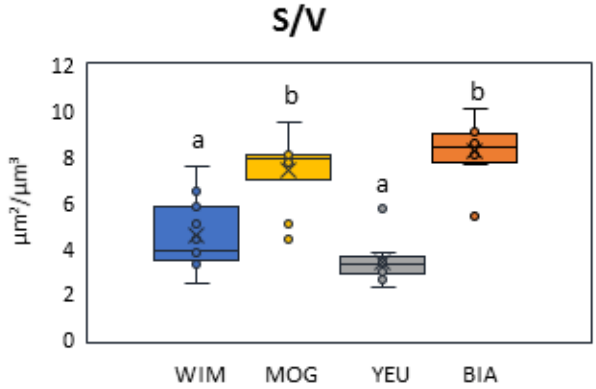
| Primers | Sequences |
|---|---|
| *GLU-5'* Fw | 5'-CCAGTATACAAACCTGTGAAGA-3' |
| *GLU-5'* Rv | 5'-TGTTGTCTTAATAGGTTTGTAAGA-3' |
| SNP-001 Fw *M. edulis* | 5'-CGCCTATGCCACCAACGACA-3' |
| SNP-001 Fw *M. galloprovincialis* | 5'-CGCCTATGCCACCAACGACT-3' |
| SNP-001 Rv | 5'-GGGATAACCCCTGGAATGTT-3' |
| SNP-064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

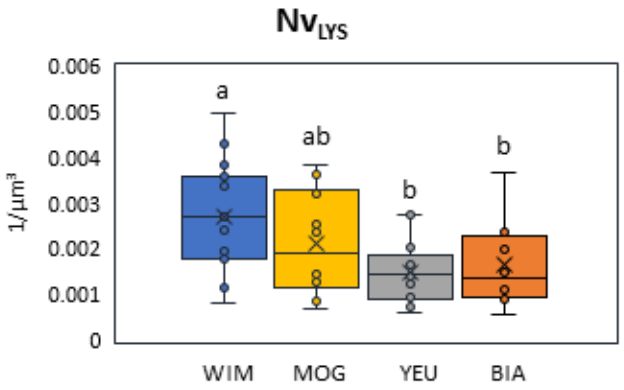
The results for the three genetic markers studied on the 4 groups are given in percentage (N=25). WIM, Northern France: *M. edulis*; MOG, Northern Brittany: mainly Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque cost: mainly Atlantic *M. galloprovincialis.*

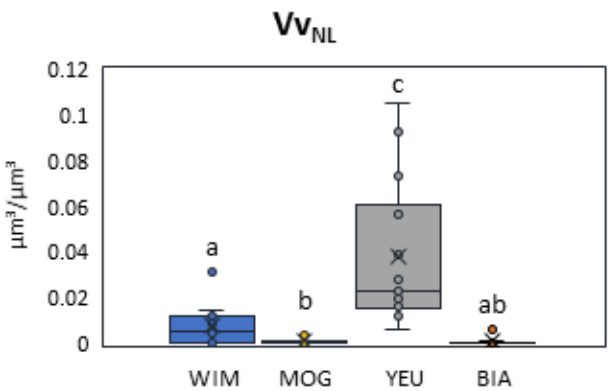
| | WIM | | | | BIA | | | | MOG | | | | YEU | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GLU-5' | SNP-001 | SNP-064 | average | GLU-5' | SNP-001 | SNP-064 | average | GLU-5' | SNP-001 | SNP-064 | average | GLU-5' | SNP-001 | SNP-064 | average |
| Homozygote edulis | 100 | 48 | 84 | 77 | 4,2 | 0 | 4,4 | 3 | 0 | 4,2 | 8 | 4 | 32,2 | 20,2 | 44 | 32 |
| Homozygote galloprovincialis | 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 |

**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.**



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

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 Atlantic *M. galloprovincialis* introgressed with *M. edulis*; YEU, West coast: hybrid zone; BIA, Basque coast: mainly Atlantic *M. galloprovincialis.* The standard deviation is given by at least 2 replicate measurements on the same sample.

PCB ng $g^{-1}$ 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 |

PBDE ng $g^{-1}$ 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 |

PAH ng $g^{-1}$ 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 |