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1 Spatial and seasonal use of biomarkers in Dreissenids: implications for
2 biomonitoring

3

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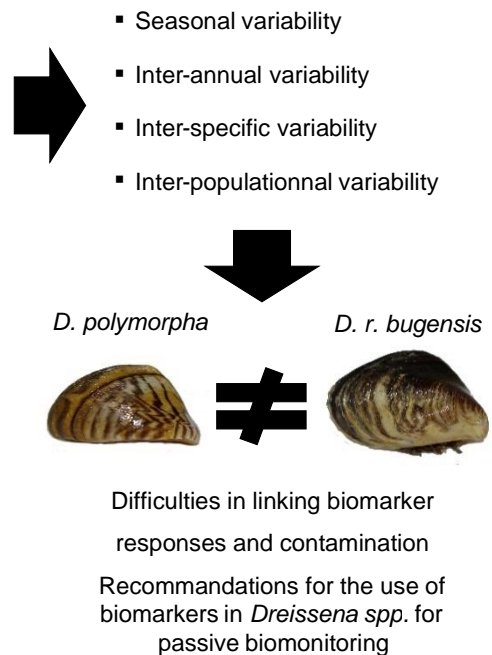
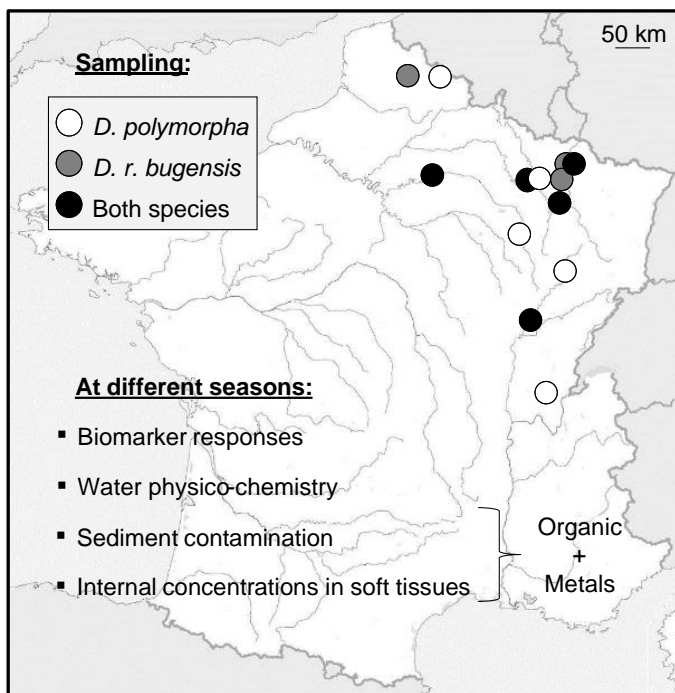
17 **Abstract**

18 In addition to pollution, organisms are exposed to natural variations of the biotic and abiotic factors of
19 their environment. A battery of sub-cellular biomarkers has been measured seasonally in several
20 populations of both *Dreissena polymorpha* and *Dreissena rostriformis bugensis*. To observe and
21 understand the variability associated with biomarker responses, water physico-chemistry, sediment
22 contamination and internal concentrations of contaminants in soft tissues were also considered. Results
23 evidenced seasonal, inter-specific and inter-population variability of the measured responses,
24 highlighting the needs (1) to acquire long-term data on the studied populations and (2) to incorporate
25 environmental parameters and contamination in the interpretation of biological responses. From a
26 biomonitoring perspective, significant relationships were identified between biomarkers, internal
27 concentrations of contaminants in soft tissues and sediment contamination in *D. r. bugensis* and, to a
28 lesser extent, in *D. polymorpha*. The detailed interpretation of each biomarker of the battery measured
29 is complex, but a global analysis of all biomarkers at once allows to obtain this signature of the
30 contamination of the studied sites.

31

32 **Graphical Abstract**

33
34



35 **Keywords**

36 *Dreissena polymorpha*, *Dreissena rostriformis bugensis*, passive biomonitoring, confounding factors,
37 variability, freshwater bivalve, biomarkers.

38 **Highlights**

- 39 • Within population variability decreases in winter, while between population variability remains
40 constant
- 41 • Biomarkers ability for discriminating among populations is stable throughout the year
- 42 • *Dreissena polymorpha* exhibits highly active defence responses and energy metabolism
- 43 • *Dreissena r. bugensis* shows high level of reserves together with higher levels of damages
- 44 • *Dreissena r. bugensis* seems to be the more suitable species to reveal sediment contamination
45 in a passive sampling design

46 1. Introduction

47

48 Dreissenid mussels, and particularly *Dreissena polymorpha*, are often used for *in situ*
49 monitoring using biomarker responses (de Lafontaine et al., 2000; Palais et al., 2012, Binelli et al. 2015).
50 Most of these studies are focused on the impact of pollution on biomarker responses, to see if biomarkers
51 are able to reflect contamination levels (Faria et al., 2014; Pain-Devin et al., 2014; Parolini et al., 2013).
52 However, the biological changes observed in an organism are not solely due to the presence of
53 contaminants. Apart from contamination, organisms are simultaneously subjected to natural seasonal
54 variations in biotic (*e.g.* reproductive cycle, food availability) and abiotic factors (*e.g.* temperature,
55 salinity) which may also influence biomarkers. If these factors are not considered, the effect of
56 contaminants on biomarkers can easily be misestimated (Forbes et al., 2006; Hagger et al., 2006).

57 The most studied environmental variable is probably temperature. Temperature influences
58 biomarkers in a number of ways (Sokolova and Lannig, 2008), including modulating enzyme activities
59 or contaminant toxicokinetics and toxicodynamics, and it can even lead to the death of organisms when
60 it is too high (Del Piero et al., 2012). Other factors also come into play such as light, circadian cycle,
61 diet, pH, oxygen content, changes in habitat, predation, parasitism, transition from one life stage to
62 another, sex, age, sexual maturity and reproductive status. All of these factors may act directly, through
63 the generation of Reactive Oxygen Species (ROS) for instance, or indirectly by modulating food supply
64 and energy balance between the different biological functions. Wojtal-Frankiewicz et al. (2017)
65 highlighted the impact of seasonality and of several abiotic factors (especially ionic strength, oxygen,
66 and pH) on oxidative stress in *D. polymorpha*, suggesting limited tolerance to changing environmental
67 conditions.

68 The role of these factors on biomarker levels can be studied both in the laboratory and in the
69 field. Laboratory studies can refine and enable to understand the mechanisms responsible for observed
70 ecological effects, but usually treat these factors separately (Johnston and Roberts, 2009). Thus, for
71 dreissenids (and other species), studies under multiple stress conditions are quite limited in the
72 laboratory and are rather carried out in the field. Some *in situ* studies have focused on the seasonality of
73 measured responses (Fanslow et al., 2001; Pain et al., 2007; Stoeckmann and Garton, 1997) or on the
74 impact of other factors on biomarkers such as reproductive status (Lazzara et al., 2012) or water physico-
75 chemistry (Pain et al., 2007; Wojtal-Frankiewicz et al., 2017).

76 Contrary to most of laboratory studies, *in situ* experimentations are often conducted on several
77 populations (Binelli et al., 2005; Faria et al., 2014; Parolini et al., 2013), and sometimes imply
78 transplanted organisms (active biomonitoring; Châtel et al., 2015; Guerlet et al., 2007, Catteau et al.
79 2022). In these cases, in addition to variations related to the above-cited parameters, responses may also
80 vary from one population to another due to local adaptation (Becker van Slooten and Tarradellas, 1994;
81 Berthet et al., 2011; Bervoets et al., 2004). Indeed, each population sets up specific mechanisms
82 depending on its local environmental conditions and its genetic characteristics, then expressing specific

83 biological responses (Millward and Klerks, 2002, Potet et al. 2018b). To properly interpret biomarkers,
84 it must be kept in mind that the response measured at a given time is the result of a one-off reaction to
85 a given event combined with a reaction shaped by the environment and genetics over time through
86 acclimatisation and/or adaptation mechanisms. Then inter-population variability of biomarkers should
87 be considered with caution.

88 When it comes to dreissenids, inter-specific differences must also be accounted for. Indeed,
89 French hydrosystems have long hosted *Dreissena polymorpha* and have now been colonised by
90 *Dreissena rostriformis bugensis* for about fifteen to twenty years (Heiler et al., 2013; Marescaux et al.,
91 2015). Whereas most studies dealing with both species were focused on evaluating their habitat
92 preference to predict their invasion range (Baldwin et al., 2002; De Ventura et al., 2016; Ellis and
93 MacIsaac, 2009), the question raises about the use of the newly arrived species in biomonitoring studies
94 in addition to the use of *D. polymorpha*, the traditional model species in freshwaters ecotoxicology for
95 which a large body of data is already available. In recent years, several studies have shown differences
96 in biomarker responses between the two species (Evariste et al., 2018; Kerambrun et al., 2018; Potet et
97 al., 2018a; Schäfer et al., 2012) or not (Farkas et al., 2017). Thus, inter-specific variability in biomarker
98 responses between *D. polymorpha* and *D. r. bugensis* should also be evaluated.

99 Comparisons are often conducted between several populations of one species (inter-population
100 variability; see Pain-Devin et al., 2014 for *D. polymorpha*; Ács et al., 2016 for *D. r. bugensis*) or between
101 the two species (inter-specific variability; Kerambrun et al., 2018; Schäfer et al., 2012), but rarely on
102 several populations of both species on a large geographical scale. In this paper, we gathered all the
103 biomarker data obtained during passive biomonitoring campaigns on several populations of both *D.*
104 *polymorpha* and *D. r. bugensis* at a large geographical scale in north-eastern France. A battery of 11
105 sub-cellular biomarkers was measured, representative of antioxidative mechanisms, energy metabolism
106 and cellular damages. Physicochemical parameters, sediment contamination and contaminant
107 bioaccumulation were also recorded. The aims were (1) to study biomarker responses according to
108 seasonal and spatial variations (*i.e.* in different populations at different seasons), (2) to compare
109 biomarker responses between the two species, and (3) to evaluate the influence of physicochemistry and
110 environmental contamination on biomarker responses, in order to identify what drives the variability of
111 *in situ* biomarker responses and which environmental factors could lead to a misreading of biological
112 responses. This paper also seeks to describe and understand the variability of responses observed
113 between the two species, and between different populations. In addition, we will show that it is possible
114 to consider the multiple factors of variation occurring in natural environments, even if understanding
115 and analysing the whole dataset from a statistical point of view remains challenging.

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117 **2. Material and methods**

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119 **2.1. Sampling sites**

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Dreissenid mussels were collected in October 2015, April 2016 and February 2017 from thirteen sites, located mainly in north-eastern France (Figure 1).

Two sites were located in the North. **No1** (Courcelles-lès-lens; *D. r. bugensis* only) is a former harbour on the Deûle waterway which was rehabilitated in a leisure base, and is located between two factories known for their past releases of lead, cadmium and zinc. **No2** (Condé-sur-l'Escaut; *D. polymorpha* only) is a pond located in a former coal mining area.

In the Paris Basin, **Oi1** (Verneuil-en-Halatte; both species) is located on the Oise river (used for commercial shipping), downstream a waste water treatment plant.

Six sites were located in the North-East. Four of them cover approximately 60 km of the Moselle River, a tributary of the Rhine River used for commercial shipping, submitted to strong industrial and urban influence. **Mo1** (Montigny – canal de Jouy; both species) and **Mo2** (Montigny – canal de Metz; *D. r. bugensis* only) are two adjacent waterways located in the Metz agglomeration. Mo1 is located on the canalized Moselle; Mo2 corresponds to the derivation of the Moselle river. Upstream, there is a wood treatment plant using metal salts and creosote, and also a former tar refinery, closed in 1996 (PAHs, phenols, naphthalene, creosote). **Mo3** (Jouy aux arches; *D. r. bugensis* only) is also located on the canalized Moselle (canal de Jouy), but slightly upstream compared to Montigny. **Mo4** (Liverdun; *D. r. bugensis* and in 2017 also *D. polymorpha*) is located even more upstream, before the confluence with the Meurthe River. In North-East, two supplementary sites were located in the Meuse basin. On **Me1** (Saint-Mihiel; both species), mussels were sampled on the walls of a lock (thus they are frequently subject to emersion) on the canal de l'Est branche Nord, which is dedicated to recreational activities (boating, shipping, fishing) in spring and summer. **Me2** (Madine; *D. polymorpha* only) is a lake fed by a tributary of the Moselle. It is a leisure base, prohibited to thermal motor boats, which constitutes a reserve of drinking water for the city of Metz. To our knowledge, there is no particular source of pollution on Me1 and Me2.

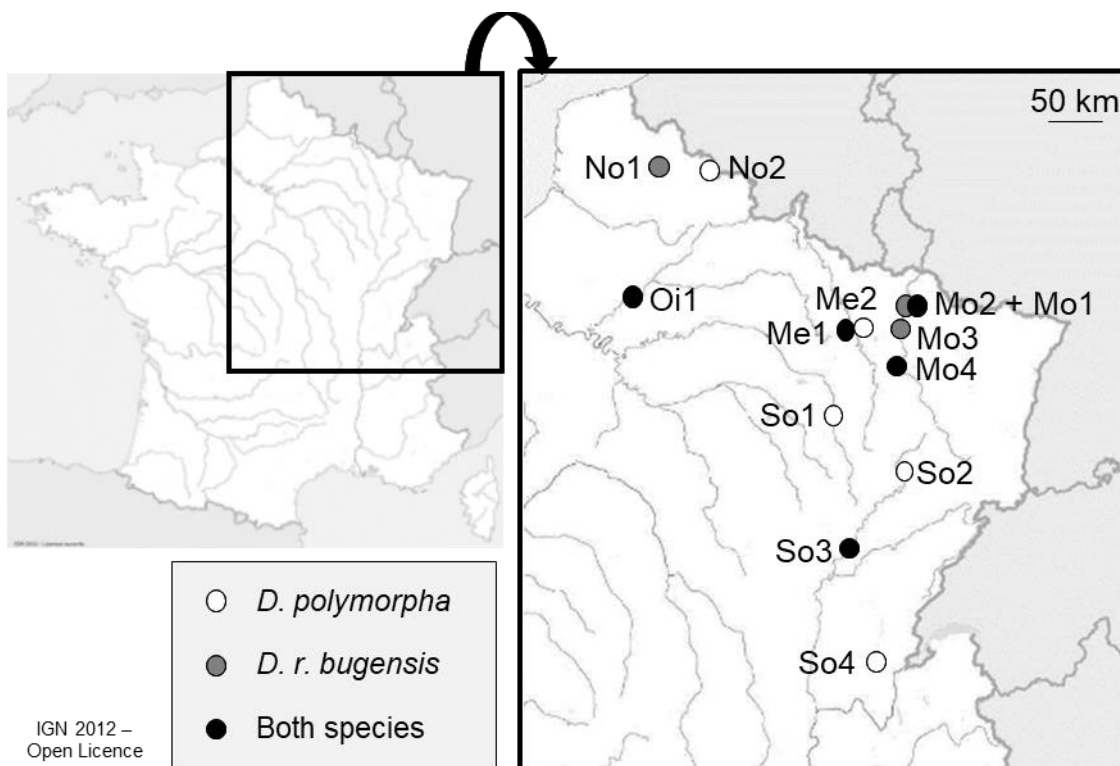
Going down to the South we had four more sites. **So1** (Riaucourt – canal entre Champagne et Bourgogne; *D. polymorpha* only) is a larger waterway used for commercial shipping, frequently emptied and where the water level often fluctuates. **So2** (Scey-sur-Saône; *D. polymorpha* only) is a marina located on the Saône. **So3** (Seurre; *D. r. bugensis* and in 2017 also *D. polymorpha*, discovered on pontoons only, in very low abundance) is a harbour, between the Saone and its diversion channel. A boat sank on September 2015, leading to a release of fuel within the harbour. **So4** (Nantua; *D. polymorpha* only) is a lake of glacial origin, classified as a protected natural site since 1936.

The Table A.1. gives a descriptive summary of these sampling sites.

2.2. Mussel collection

156 On each site, organisms dedicated to biomarker measurements were hand-collected by section
 157 of their byssus (n = 12 for each population). The two species were differentiated according to
 158 morphometric parameters (Pavlova and Izyumov, 2014). Species identification in the field was further
 159 confirmed by zymograms of Cu/Zn-SOD which allows discrimination of the two species (Figure A.1.;
 160 Rocher et al, 2020). Just after sampling, organisms were measured (length, width, height of shell). The
 161 mean shell length was 22 ± 2.9 mm for *D. polymorpha* populations and 25.2 ± 3.9 mm for *D. r. bugensis*.
 162 The volume of the shell, calculated as the product of length, width and eight, was higher for *D. r.*
 163 *bugensis* populations (5.3 ± 2.2 cm³) than for *D. polymorpha* ones (2.9 ± 1.1 cm³).

164 Immediately after morphometrics measurement, and directly in the field, the digestive gland of
 165 each mussel was dissected, frozen in liquid nitrogen and kept at -80 °C until biomarker measurements
 166 in the laboratory.



167
 168 *Figure 1: Sampling sites in north-eastern France and species occurrence (detailed description in*
 169 *Table A.1.)*

170
 171 **2.3. Biomarker measurements**

172
 173 Biomarker responses were measured in digestive gland as it is considered as the most
 174 metabolically active organ in mussels (Viarengo et al., 2007). We used an automated spectrophotometer
 175 (Konelab 20-XTi, ThermoScientific) that operates on very small volumes and allows the measurement
 176 of all biomarkers on each individual digestive gland. The detailed methods used for crushing, differential
 177 centrifugation and biomarker measurements are available in Garaud et al. (2016) and Potet et al. (2018a).

178 Briefly, two sets of measures were performed for each digestive gland. On the crude extract
 179 (after crushing and centrifugation at 250 x g), we measured the concentrations of proteins (ProtB),

180 triglyceride (Tri), cholesterol (Chol) and lipid hydroperoxide (LOOH), and the activity of electron
181 transport system (ETS) and acid phosphatase (ACP). On the cytosolic fraction (after an additional
182 centrifugation at 20 000 x g), the activity of glutathione-S-transferase (GST), lactate dehydrogenase
183 (LDH) and caspase-3 (Csp) the total antioxidant capacity (TAC) and the concentrations of proteins
184 (ProtFC) were measured.

185

186 **2.4. Sediment contamination, internal concentrations and water physico-chemistry**

187

188 Concerning sediment contamination, 3 points were sampled per site. The 5 first centimetres of
189 the sediment layer were sampled with a plastic shovel on the river bank, with a distance of 1m between
190 each sample, surrounding the dreissenids sampling point. The three resulting samples were then mixed
191 and 500 g fractions were sent for metals, PCBs and HAPs analysis to La Drome Laboratoire (Cofrac
192 accreditation n° 1-0852). Sediments were mineralized according to EPA 3052 method (using
193 hydrofluoric and nitric acids). Total contaminant concentrations were measured on the <2 mm fraction,
194 using gas chromatography-mass spectroscopy (GC-MS) for HAPs and PCBs (French norm XP X 33-
195 012), inductively coupled plasma atomic emission spectroscopy (ICP-AES) for Al, Fe, Mn, Zn (EN ISO
196 11885), inductively coupled plasma mass spectroscopy (ICP MS) for As, Cd, Cr, Co, Cu, Ni, Pb (EN
197 ISO 17294-2) and cold vapor atomic fluorescence spectrophotometry for Hg (EN ISO 17852).

198 In February 2017, 20 organisms were also sampled on each site (when possible) to measure
199 internal concentrations of contaminants. For each site, mussels were dissected *in situ*, total soft tissues
200 (without byssus) were pooled and frozen in liquid nitrogen. In the laboratory, pools were defrosted and
201 dried in an oven at 60 °C for 48 h, weighed, and sent to Micropolluants Technologie S.A. for analysis
202 (Cofrac accreditation n° 1-1151). Gas chromatography-mass spectroscopy (GC-MS) was used for HAPs
203 and PCBs (EN ISO 15753), inductively coupled plasma mass spectroscopy (ICP-MS) was used for
204 metals, except Hg which was measured using cold vapor atomic fluorescence spectrophotometry (EN
205 ISO 17852).

206 Temperature, oxygen saturation, conductivity and pH were measured *in situ* using a Multi 3430
207 SET G probe (WTW®), and 2 L of water were also sampled in polypropylene bottles and stored at 4°C
208 for further measurements in laboratory. Total suspended matter (TSM) and volatile suspended matter
209 (VSM) were determined after water filtration and differential weighing of the filter before and after
210 drying at 105 °C. VSM corresponds to the part of TSM volatilized after calcination of the filter at 525
211 °C. Nitrogen compounds were measured by ionic chromatography (NO₃) or absorption spectrometry
212 (NH₄). Phosphates (PO₄) and total phosphorous (P_{tot}) were measured using absorption spectrometry and
213 chlorine (Cl) and sulphates (SO₄) concentrations by ionic chromatography. Other ions (Ca, Mg, Na, K)
214 were measured by flame atomic absorption spectrophotometry. Total anions (TotA), cations (TotC) and
215 ionic balance (IonBal) were calculated depending on ion concentrations. Water hardness (WH) was
216 measured by neutralizing a volume of water with a strong mineral acid. Chemical oxygen demand

217 (COD) was measured by adding potassium dichromate in an acid medium, to oxidize organic materials.
 218 The excess in dichromate was then titrated with a solution of iron(II)sulfate and ammonium. Biological
 219 oxygen demand (BOD) was measured by monitoring the decrease in oxygen concentration in the sample
 220 between 0 and 5 days.

221

222 2.5. Data analysis strategy and statistical analysis

223

224 Measurement of the above-cited parameters was not carried out at each campaign, and the
 225 biomarkers could not be measured for each species, each population and during all the sampling
 226 campaigns, due to variable availability of organisms along the study. Table 1 summarizes the parameters
 227 measured for each population. From this table follows the data analysis strategy, the resulting dataset
 228 being subdivided according to the question we addressed.

229 *Table 1: Sampling plan of populations of D. polymorpha and D. r. bugensis in situ. The blue cells*
 230 *represent the biomarker data collected, the grey cells represent the data related to contamination*
 231 *(environmental and internal concentrations).*

Site	October 2015				April 2016			February 2017					
	Biomarkers		Sediments contamination	Physico-chemistry	Biomarkers			Biomarkers					
	<i>D. polymorpha</i>	<i>D. r. bugensis</i>			<i>D. polymorpha</i>	<i>D. r. bugensis</i>	Physico-chemistry	<i>D. polymorpha</i>	<i>D. r. bugensis</i>	Sediments contamination	Physico-chemistry	Internal concentrations	
Me1	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
Me2	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
Mo1	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
Mo2	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
Mo3	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
Mo4	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
No1	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
No2	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
Oi1	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
So1	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
So2	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
So3	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey
So4	Blue	Blue	Grey	Grey	Blue	Blue	Grey	Blue	Blue	Grey	Grey	Grey	Grey

232

233

234 Based on this table of acquired results, we treated the data as follows:

235 - inter-specific differences were studied by considering only sites where the two species co-
 236 occurred on a given sampling date to avoid other sources of variability (Me1, Mo1, Mo4, So3,
 237 Oi1)

238 - seasonal variability was studied separately for the two species, on the sites where individuals
 239 could be sampled at all of the three seasons: Me1, Me2, Mo1, No2, Oi1, So1, So2 and So4 for
 240 *D. polymorpha* and Mo1, Mo3, Mo4, No1 and So3 for *D. r. bugensis*

241 - relationships between biomarkers values and sediment contamination were investigated in
 242 October 2015 and February 2017, and with internal concentrations only in February 2017, on
 243 every sampled site.

244 Concerning statistical analysis, the influence and potential interactions of environmental factors
245 (site and date) on biomarker responses was analysed for each species through MANOVA. Normality
246 (energy package) and homoscedasticity were previously tested.

247 Principal Component Analyses (PCA) were performed to evidence inter-specific and seasonal
248 variability in biomarker responses. Similarity of patterns was assessed by co-inertia analysis. They are
249 based on the crossing of 2 PCAs containing the same number of rows. They are carried out using the
250 "coinertia" function of the ade4 package. A Monte-Carlo test ("randtest" function) then makes it possible
251 to determine if the co-inertia is significant, that is if there is a common structure between the two
252 datasets. These analyses were made using R software (R Development Core Team, version 3.3.0). A
253 threshold of $p \leq 0.05$ was considered significant.

254 The final part of the results will aim to relate biomarker responses to physico-chemical
255 parameters, internal concentrations of contaminants in organisms and sediment contamination. Partial
256 Least Square (PLS) regressions were performed using Tanagra software (version 1.4). PLS regression
257 aims to predict the values taken by a group of Y explained variables from a set of X explanatory
258 variables. It makes it possible to process a large number of descriptors simultaneously, even when the
259 information they provide is redundant. For all the PLS, the values were systematically retained on the
260 first 3 factors. VIPs (Variable Importance in the Projection) indicate the relative importance of
261 biomarkers that explain contamination. Here we have retained only the VIP computed on the first three
262 factors. Significance thresholds were arbitrarily set at 60% for the cumulated explained variance, at 0.8
263 for cumulated R^2 and at 1 for VIP.

264

265 **3. Results and discussion**

266

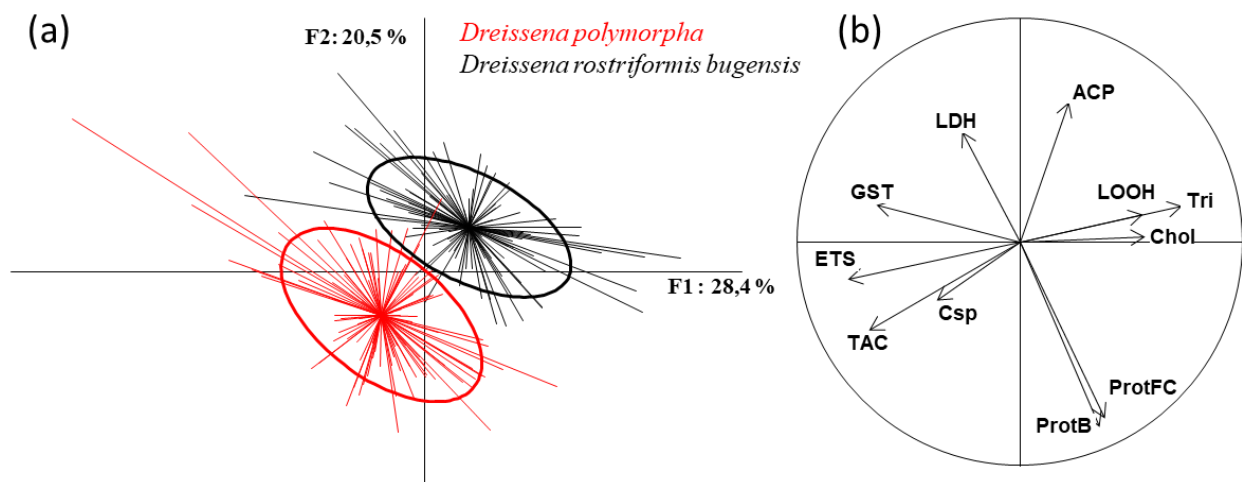
267 **3.1. Inter-specific variability**

268

269 Both species could be sampled at the same time in nine occasions: in October 2015 on the sites
270 Me1, Mo1, in April 2016 on the sites Me1, Mo1 and Oi1 and in February 2017 on the sites Mo1, Mo4,
271 Oi1 and So3. The detailed results (mean \pm standard deviation) for all sampling dates and sites are
272 presented in Table A.2.

273 The PCA shown in figure 2 describes all the responses obtained for each species, all sites and
274 dates combined. It reveals a clear difference in the pattern of response between the two species. To
275 appreciate the extent of this difference, it is important to realize that the figure integrates, for each
276 species, several sources of variability: three dates over three years and several populations sampled in
277 different hydromorphological and chemical contexts. Despite this, the ellipses depicting the responses
278 of the 11 biomarkers for each of the two species are distinct. The inter-species differences in the pattern
279 of biomarkers response are therefore much larger than those generated by the seasonal effect and/or the
280 population effect.

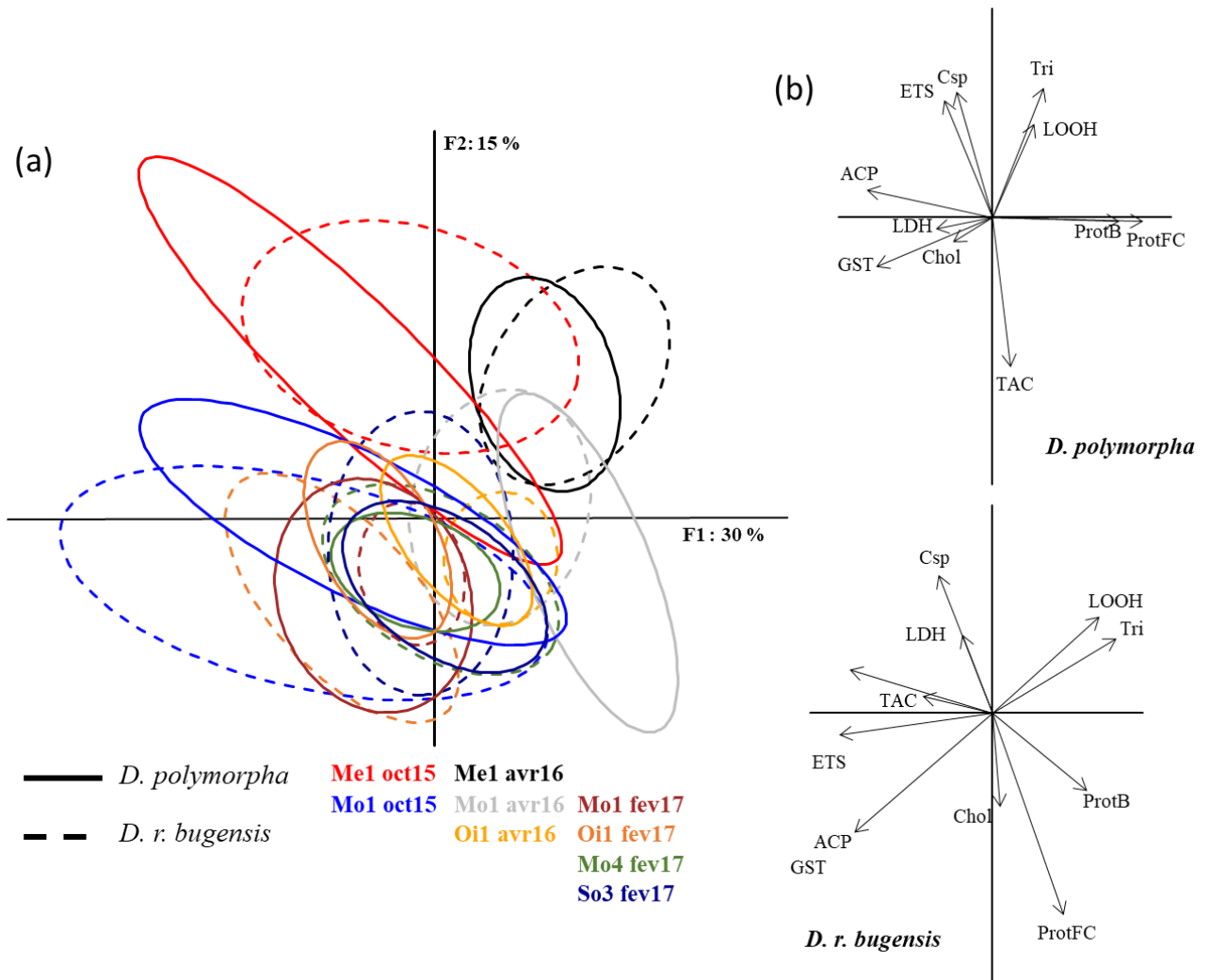
281 *D. r. bugensis*, on the F1 axis, generally has lower antioxidant and antitoxic responses (TAC,
 282 GST) than *D. polymorpha*, what may reflect a lower protection against cellular damages and then
 283 explain the higher lipid peroxidation (LOOH) that is observed in *D.r. bugensis* compared to *D.*
 284 *polymorpha*. Another study has also evidenced more damages for *D. r. bugensis* upon exposure to
 285 pesticides during laboratory experiments (Schäfer et al., 2012). During our survey, *D. r. bugensis*
 286 displays more energy reserves (Tri) but a lower activity of the mitochondrial ETS than *D. polymorpha*.
 287 Together these results reveal a clear discrepancy between both species in terms of metabolic and defence
 288 strategies. Differences in biomarker levels between both species have already been evidenced in our
 289 previous studies (Potet et al., 2018a,b). These inter-specific differences, observed *in situ* in the present
 290 work, were maintained during laboratory exposures, with, for example, a higher nickel tolerance for *D.*
 291 *polymorpha* populations (Potet et al., 2018b), and a better capacity for this species to activate its defence
 292 mechanisms in the presence of the metal and to respond gradually to stressors (Potet et al., 2016).
 293 Discrepancies between the responses of both species were also recorded by other authors, about reactive
 294 oxygen species regulation and haemocyte oxidative activity (Evariste et al., 2018), expression of genes
 295 involved in metabolic activities and defence mechanisms (Kerambrun et al., 2018) and energy allocation
 296 processes (Louis et al., 2018).



297
 298 *Figure 2: Inter-specific variability of biomarkers for populations of the two species sampled in October*
 299 *2015, April 2016 and February 2017. The analysis was performed on the table individuals x biomarkers*
 300 *(216 individuals x 11 biomarkers). (a) Individual plotted on the F1F2 factorial plane and grouped*
 301 *according to the species. (b) correlation circles of the 11 biomarkers on the axis F1F2.*

302 As the two studied species present significantly different patterns of response for some
 303 biomarkers, we chose to perform a co-inertia analysis between the two PCA computed independently
 304 for each species in order to compare their response patterns to environmental gradients. Projection of
 305 individuals on the common factorial plane (Fig. 3a) evidenced similarities of relative positions of
 306 samples, grouped according to the site and the season, that were confirmed by a permutation test
 307 ($p=0.001$). The comparison of variable projection evidenced similar patterns for only two biomarkers,
 308 GST and Csp (Fig 3b). Thus, although biomarker levels and trends are different between *D. polymorpha*

309 and *D. r. bugensis*, both species allow the discrimination of the studied sites and seasons in the same
 310 way.

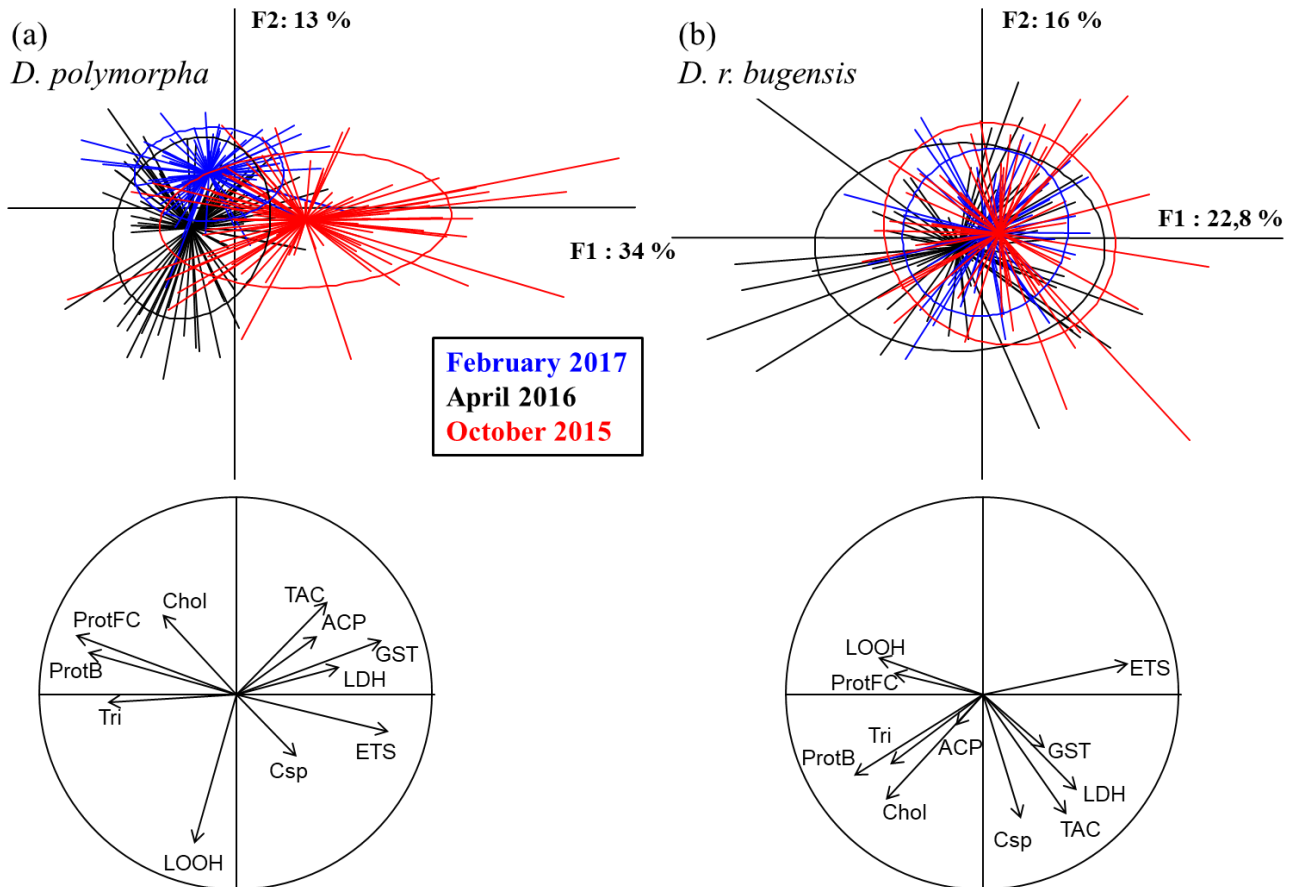


311
 312 *Figure 3. Inter-specific comparison of biomarkers for populations of the two species sampled in October*
 313 *2015, April 2016 and February 2017 on the same sites. The co-inertia was performed on the table*
 314 *individuals x biomarkers (216 individuals x 11 biomarkers for each species). The factorial plane F1F2*
 315 *(Figure 3a) represents the projection of individuals of the two species on the same space. Variables*
 316 *projected on the associated correlation circles (Figure 3b) can be compared directly between species.*
 317

318 3.2 Seasonal variations

319
 320 Seasonal variations in biomarker responses are presented (all sites together) on Figure 4. Figure
 321 4a highlights biomarker variations as a function of the season, with a shift of responses from one season
 322 to another for *D. polymorpha*. The partitioning of the variance indicates that the date and the site explain
 323 38% of the observed variability, with 62% of unexplained variance. The MANOVA shows that site,
 324 date and their interaction influence significantly biomarker responses ($p < 2.2 \cdot 10^{-16}$ in all cases). The
 325 correlation circle associated with this PCA reveals that the energy reserves (proteins, triglycerides,
 326 cholesterol) are higher in April and February than in October. In addition, in October, energy metabolism
 327 is more active (LDH, ETS), as is the activity of defence markers (GST, TAC). These seasonal variations

328 are in accordance with the reproduction cycle involving an increase of reserves storage during spring in
 329 order to produce gametes. Then after the gamete release in summer, energy reserves are low in autumn
 330 and the metabolic activity is dedicated to the maintenance and defence to prepare the winter rest phase.
 331 The Figure 4a also shows that in winter (February 2017), the size of the ellipse is very small compared
 332 to other dates meaning either a lower interindividual or a lower inter-population variability.
 333



334
 335 *Figure 4: Temporal variability of biomarker responses for D. polymorpha (a) and D. r. bugensis (b)*
 336 *populations at different seasons. F1F2 factorial design of the array individuals x biomarkers and*
 337 *associated correlation circles. Figure 4a: No2, Oi1, Mo1, Me1, Me2, So1, So2 and So4 populations of*
 338 *D. polymorpha in October 2015, April 2016 and February 2017 (288 individuals x 11 biomarkers).*
 339 *Figure 4b: No1, Mo1, Mo3, Mo4 and So3 populations of D. r. bugensis in October 2015, April 2016*
 340 *and February 2017 (180 individuals x 11 biomarkers).*
 341

342 The same analyses were carried out for *D. r. bugensis* (Figure 4b). They indicate that the date
 343 and the site explain 31% of the observed variability, with 69% of unexplained variance. The MANOVA
 344 shows that site, date ($p < 2.2 \cdot 10^{-16}$ in both cases) and their interaction ($p = 1.1 \cdot 10^{-11}$) influence
 345 significantly biomarker responses. We observed less seasonal variations than for *D. polymorpha*, but a
 346 common feature is the reduced size of the ellipse in February compared to that of April and October.

347 To assess more thoroughly the source of variability at each season, a between-class analysis was
 348 performed on each PCA. It allows to calculate the proportion of variance induced by between-site
 349 differences and to assess inter-individual variation within each site. For *D. polymorpha*, the site

350 contributes for 33.3%, 29.2% and 30.8% of the total variance for October 2015, April 2016 and February
 351 2017 respectively. For *D. r. bugensis*, the site contributes for 21%, 23% and 25% of the total variance
 352 for October 2015, April 2016 and February 2017 respectively. Thus, the proportion of the variance
 353 explained by the site effect is not dependant of the season and the reduction of ellipses inertia in February
 354 can be attributed to a decrease in within-population (*ie* between individuals) variability. Co-inertia
 355 analysis confirmed that both species exhibited similar patterns of biological responses at the three
 356 seasons (Table 2).

357

358 *Table 2: Results of the 6 co-inertias performed to study the co-structures between sampling dates.*
 359 *Each co-inertia is based on the crossing of two PCAs. Significance of co-inertia (that is, revealing a*
 360 *structure common to both datasets) are based on a permutation test.*

	<i>D. polymorpha</i>	<i>D. r. bugensis</i>
October 2015 vs April 2016	0.001	0.012
October 2015 vs February 2017	0.001	0.03
April 2016 vs February 2017	0.001	0.004

361

362 To sum up and conclude, seasonal variations in biomarker responses were observed for both
 363 species. In addition to contaminant fluxes, which may themselves fluctuate, seasonal variations in
 364 biological responses can be partly explained by fluctuations in environmental factors (*e.g.* temperature,
 365 conductivity, oxygenation). Temperature and food quantity play a very important role in the metabolism
 366 of ectotherm organisms. As shown in previous studies (Leiniö and Lehtonen, 2005; Potet et al., 2018a),
 367 an increase in temperature generally leads to an increase in metabolic demand. If combined with a
 368 lowered food availability (in winter and spring), we can expect that dreissenids will have less energy to
 369 allocate to their defence mechanisms.

370 These seasonal variations could also be closely related to biotic factors such as size, weight, sex,
 371 energetic status and reproductive cycle. Faria et al. (2014) showed, for example, that reproductive status
 372 in different populations of *D. polymorpha* was strongly correlated with the responses of some
 373 biomarkers (superoxide dismutase, catalase, GSH, GST, multixenobiotic resistance, LDH, lipid
 374 peroxidation, DNA strand breaks). Guerlet et al. (2007) also showed the seasonality of biological
 375 responses in *D. polymorpha*, related to the reproductive cycle, with an increase in lipid content and
 376 filtration activity during the reproductive period, as the temperature increases. At a lower level of
 377 organization, the evolution of the reproductive status of organisms over the seasons also leads to a
 378 modification of the transcriptome in *D. polymorpha* (Navarro et al., 2013). Organisms thus undergo
 379 strong metabolic modifications according to the seasons, in connection with their reproductive status. It
 380 may explain why our study reveals that, in field conditions, the lowest level of reserves was observed in
 381 October. Thus, autumn could be a season where organisms are particularly sensitive to an additional
 382 stress.

383 Biomarker variations can also be related to the ecology of organisms (parasitism, predation,
384 competition). As example, parasite prevalence in *D. polymorpha* follows a seasonal dynamic (Minguez
385 and Giambérini, 2012), and parasites influence biomarkers (Minguez et al., 2009). Another example is
386 intra-specific competition for food, which might be more important during the larval fixation period
387 (Wacker and Von Elert, 2008).

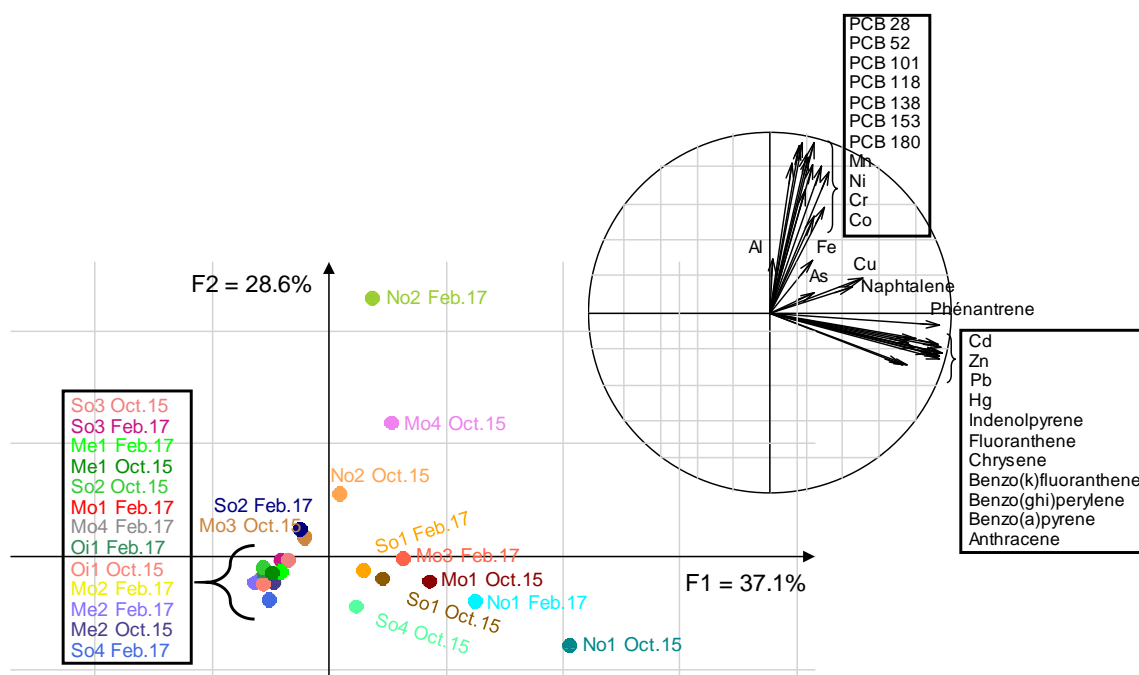
388 However, despite all these factors of variations, we evidenced that, even if raw values and range
389 of variations for the measured biomarkers differed between seasons, they are able to depict the same
390 trends in between-site variations. The decrease in inter-population variability in February for both
391 species was also observed when studying seasonal variations. It can be explained by the metabolic rest
392 for these species in winter, which tends to homogenize the responses between the different individuals.
393 It indicates that winter could be ideal for biomarker deployment, in view of a comparative approach of
394 biomarker responses between several populations; indeed, site discrimination would be more efficient
395 at this time, with a better signal/noise ratio. Such a strategy can also lead to sample less individuals to
396 achieve the same site discrimination.

397

398 **3.3. Contamination and physico-chemistry of the studied sites**

399 Both sediment contamination and water physico-chemical characteristics were measured
400 concomitantly in October 2015 and February 2017. Values for sediment contamination (Table A.3.) and
401 the associated PCA (Figure 5) isolate the No1 site, on the Deûle canal. This site is characterized by a

402 high load of PAHs and heavy metals (Hg, Pb, Zn, Cd, Cu ...). The No2 site on axis 2 is distinguished by
 403 a high level of PCBs. The other sites have a contamination gradient structured essentially by metals.
 404



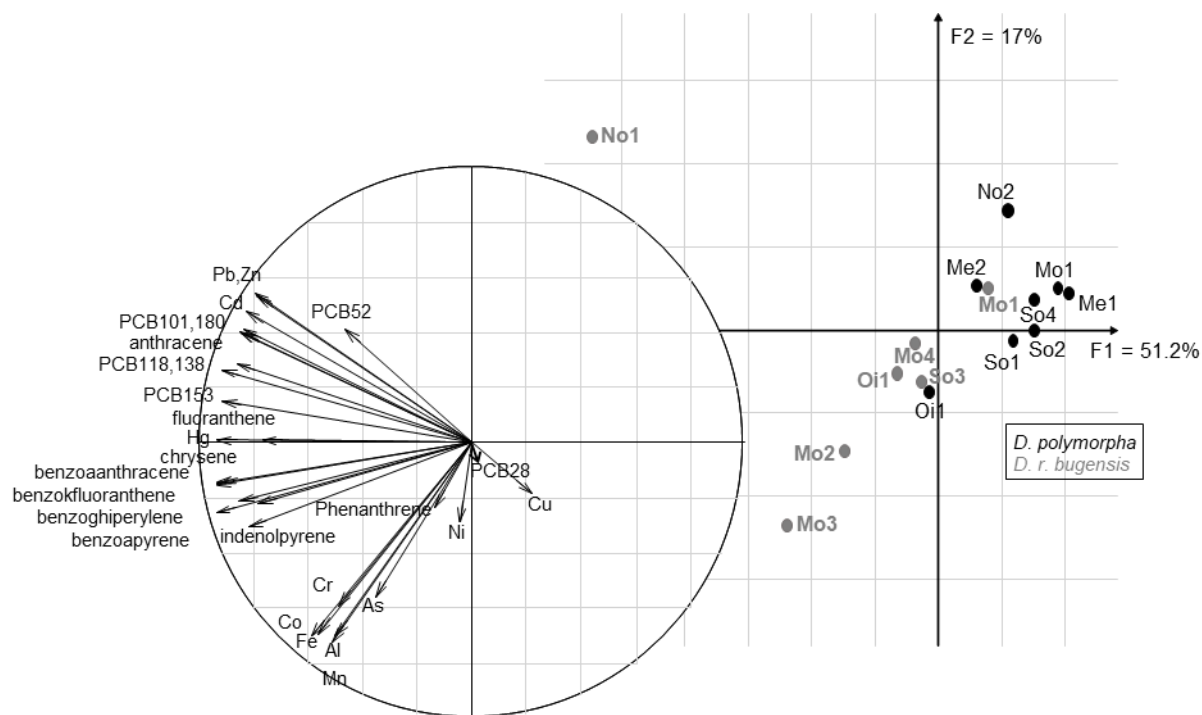
405
 406 *Figure 5.: PCA results from the sediment contamination table for the 29 contaminants measured for*
 407 *12 sites x 2 seasons (October 2015: Oct.15, February 2017: Feb.17), except for the Mo2 site analysed*
 408 *only in February 2017.*
 409

410 The other major issue from this analysis is the seasonal variability of sediment contamination.
 411 Sediments are considered to be an integrative compartment in time of environmental contamination, and
 412 analyses of sediment contamination would then reflect an average load in pollutants. But here we show,
 413 especially for No2, Mo1, Mo3 or Mo4, radical changes of the contamination profile between October
 414 2015 and February 2017. This highlights the need to carry out sediment samplings for each biomarker
 415 campaign to study finely the links between biotic and abiotic compartments.

416 The PCA performed on the physicochemical data of the water column (Figure A.3.) highlights
 417 the relative stability of these parameters at these two sampling dates (October 2015 and February 2017).
 418 The No2 site is clearly distant from the others. This pond was constructed from a former coal mine,
 419 explaining its high organic load (strong BOD), higher in autumn than in winter. The other sites present
 420 a gradient along axis 1, related to mineralization. Sites located on the Moselle river (Mo1, Mo2 and
 421 Mo3) are apart due to their high conductivity. The factorial design also highlights the great similarity,
 422 from the water chemistry point of view, of the other sampled sites. Physicochemical parameters will
 423 therefore, *a priori*, play only a marginal role in the biological response of organisms.
 424

425 3.4. Internal concentrations of contaminants in organisms

426 Internal concentrations of contaminants in organisms have been measured only in February
 427 2017 (see Table A.4. for details). The PCA (Figure 6) reveals a higher tendency to bioaccumulate both
 428 metals and organic compounds for *D. r. bugensis* populations compared to *D. polymorpha*.



429
 430 *Figure 6: PCA results from internal concentrations in 28 contaminants measured in February 2017 on*
 431 *the 15 sites where enough biological material was available.*
 432

433 These differences in bioaccumulation potential has already been evidenced in the literature for
 434 organochlorine pesticides (Schäfer et al., 2012), organic compounds (Evariste et al., 2018) and metals
 435 (Kerambrun et al., 2018; Matthews et al., 2015).

436 Such discrepancies between the two species, regarding both bioaccumulation potential and
 437 biomarker responses (see section 3.1) highlight that the declining *D. polymorpha* cannot be simply
 438 substituted by *D. r. bugensis*, and that the two species must be distinguished in ecotoxicological and
 439 biomonitoring studies.

440
 441 **3.5. Characterization of biological responses**

442 One of the aim of the present work was to relate biological responses to sediment contamination
 443 and water chemistry, in an attempt to assess those dimensions of environmental quality. As we saw
 444 earlier, the seasonal variability, both in sediment contamination and in biological responses, led us to
 445 process the data independently for both seasons (October 2015 and February 2017).

446 The ability of biomarkers to be a proxy of sediment or biota contamination was assessed via
 447 PLS (Partial Least Squares) regressions, the results of which are summarized in [Table Table-3](#) (detailed
 448 results are available in Table A.5.). Three questions were answered: is the contamination of sediments
 449 described by internal concentrations in contaminants? Is the contamination of sediments described by

450 biomarker responses? Are the internal concentrations in contaminants described by biomarker
 451 responses?

452 For both species, internal concentrations reflect the total sediment contamination, but not metal
 453 contamination. Concerning organic contaminants, accumulation in *D. r. bugensis* is more suitable to
 454 reflect PCB contamination, whereas in *D. polymorpha* it reflects HAP. In a general way, the
 455 accumulation of organic (lipophilic) contaminants by organisms reflects their presence in sediments.

456 For *D. polymorpha*, biomarker responses do neither reflect sediment contamination nor internal
 457 concentrations of contaminants in soft tissues.

458 For *D. r. bugensis*, biomarkers reflect sediment contamination and are related to bioaccumulated
 459 contaminants, except metals.

460 *Table 3: Synthesis of the PLS results concerning the relationships between internal concentration in*
 461 *contaminants and sediment contamination; biomarker responses and sediment contamination;*
 462 *biomarker responses and internal concentrations. Red crosses represent not significant PLS, green*
 463 *symbols significant PLS, yellow symbols PLS where only a particular category of contaminant*
 464 *concentrations in the sediment can be elucidated with either internal concentrations of biomarkers*
 465 *levels.*

			Explaining			
			Internal concentrations		Biomarker responses	
			<i>D. polymorpha</i>	<i>D. r. bugensis</i>	<i>D. polymorpha</i>	<i>D. r. bugensis</i>
Explained	Sediment contamination	Total	✓	✓	✗	✓
		Metals	✗	✗	✗	✓
		Organics	✓ (PAHs only)	✓ (PCBs only)	✗	✓ (PAHs only)
	Internal concentrations	Total			✗	✓
		Metals			✗	✗
		Organics			✗	✓

466

467 A lower variability was noticed in biomarker responses between *D. r. bugensis* populations than
 468 between *D. polymorpha* ones, but *D. r. bugensis* populations enable a better description of site
 469 contamination. This may seem contradictory. Our hypothesis is that *D. r. bugensis* populations are not
 470 yet adapted to their local environmental conditions, due to their more recent invasion, and thus each
 471 population responds directly to local environmental pressure.

472 On the other hand, there is a greater variability in biomarker responses between *D. polymorpha*
 473 populations, although it cannot be related with on-site contamination. Thus, considering the random
 474 sediment contamination from one year to the next (see section 3.3), we can hypothesize that (1) the
 475 historically different contamination levels between sites has led to differences between *D. polymorpha*
 476 populations, but that (2) the precise nature of the contamination being variable, populations of this

477 species responds to contamination with singular response profiles, mainly focused on defence processes,
478 which allows them to face any other conditions. In this sense, laboratory studies have shown that *D.*
479 *polymorpha* populations are more heterogeneous, more tolerant to high levels of stress, and able to
480 activate defence mechanisms when facing contamination (Potet et al., 2016; 2018a). *D. r. bugensis*,
481 recently settled, seems to be just responding to the contamination it faces at the immediate instant, and
482 collapses under severe stress.

483 Variability among populations of the same species *in situ* has also been reported for *Anodonta*
484 *anatina* (Falfushynska et al., 2016). It is recognized that the variability of organism responses may result
485 from adaptations to a wide range of environmental stressors (Lushchak, 2011), and that populations
486 living in polluted areas may develop a tolerance for pollution. Falfushynska et al. (2013) showed, for
487 example, that *A. anatina*'s response to metals depended on the origin of the mussels, as did Milan et al.
488 (2016) for *Ruditapes philippinarum*, in the lagoon of Venice. The divergence between populations of
489 *D. polymorpha* has been highlighted several times, both regarding genetic diversity (Tarnowska et al.,
490 2013), gene expression (Kerambrun et al., 2016), proteomic profiles and the expression of certain
491 subcellular biomarkers (Pain-Devin et al., 2014). Genetic differentiation is even possible for populations
492 of *D. polymorpha* very close geographically (750 m; Thomas et al., 2014). In addition to the links
493 between variation in biomarkers and contamination, the genetic composition of populations must be
494 considered as a confounding or explaining factor, which determines the response of populations to their
495 environment. The present study and those of Pain-Devin et al. (2014) and Tarnowska et al. (2013)
496 emphasize the need to know in detail the physiology and tolerance capacities of the sentinel species
497 populations used in monitoring, to reliably interpret and predict their responses to new stresses. The
498 assumptions made on *D. polymorpha* towards local adaptation do not apply for recently settled *D. r.*
499 *bugensis* populations, **since the time scale from introduction is too short to allow genetic differentiation**
500 **between populations. However, further studies are needed to understand evolution patterns and the role**
501 **of contamination in this mechanism.**

502

503 **4. Summary and conclusions**

504

505 Natural variability of biomarkers is one of the obstacles to their use in biomonitoring. Indeed,
506 the challenge of identifying the variation associated with toxic stress in a set of naturally fluctuating
507 responses is still difficult to grasp (Hanson et al., 2010).

508 In the context of the large-scale deployment of biomarkers in dreissenids, our results highlight
509 the need to pay particular attention to some points. First, the distinction between the two species (*D.*
510 *polymorpha* and *D. r. bugensis*) must be made with certainty since their responses are clearly different;
511 these two species are not interchangeable. Even if they allow for depicting the same patterns of between-
512 sites variations, the biomarkers involved are not the same. Consequently, mixing the two species in a
513 global “*Dreissena* sp.” complex may induce more within population variability and may lower our

514 capacity to identify site differences. In addition to inter-specific differences, our study points out the
515 needs: (1) to acquire long-term data on the populations of interest in order to identify specific changes
516 in the responses or temporal trends, (2) to collect data incorporating environmental parameters and
517 contamination in order to build reliable interpretation grids of biological responses depending on the
518 sampling period.

519 Here, a battery of biomarker was used, and evidenced its ability to discriminate sites on the basis
520 of sediment contamination, at least for *D. r. bugensis*, and we identified relationships between
521 biomarkers, internal concentrations in contaminants in soft tissues and sediment contamination. The
522 increase in the number of measured responses increase the complexity of data analysis and
523 interpretation, especially if we try to interpret each biomarker independently. However, we also
524 evidenced that the use of a battery strengthens the robustness of site characterization, with a stability of
525 sites signatures throughout seasons. Some efforts still need to be made to define the optimal battery, *i.e.*
526 the minimal set of biomarkers that allows to detect a modification in organisms health and to relate it to
527 the presence of environmental stressors.

528 Finally, biomarker responses are life history traits that integrate environmental factors that can
529 structure organisms physiology both through long-term trends (ie biogeographic features) or at more
530 local and/or short-term levels (ie a local pollution, local geochemical background...). A detailed
531 understanding of the relative roles of these factors acting at different time scales remains a challenge for
532 future research, and requires the implementation of observation strategies in ecotoxicology, as it is
533 already done in ecology.

534

535

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542

543 **Author contribution**

544 All authors whose names appear on the submission made substantial contributions to the conception or
545 design of the work; or the acquisition, analysis, or interpretation of data. All authors commented on
546 previous versions of the manuscript. All authors read and approved the final manuscript.

547 Devin S.: project direction, manuscript preparation, data treatment

548 Potet M.: project direction, manuscript preparation, sampling, biomarker analysis, data treatment

549 Louis F.: mussels sampling, biomarker analysis

550 Pauly D.: biomarker measurement
551 Rocher B.: species determination, manuscript preparation
552 Wagner P.: sampling
553 Giambérini L.: project direction, manuscript preparation
554 Pain-Devin S.: project direction, manuscript preparation

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561

562 **Data availability**

563 Raw data for biomarker measurements will be stored in an open data repository once the manuscript
564 accepted for publication

565

566 **Declarations**

567

568 **Competing interests**

569 The authors declare that they have no competing interests.

570

571 **Ethical approval**

572 Not applicable, the species considered in this manuscript don't need an agreement from an ethic
573 committee

574

575 **Consent to participate**

576 Not applicable

577

578 **Consent for publication**

579 Not applicable

580

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775 **Appendices**

776

777 *Table A.1.: Descriptive summary of the sampling sites (D. r. bug.: Dreissena rostriformis bugensis; D.*778 *pol.: Dreissena polymorpha)*

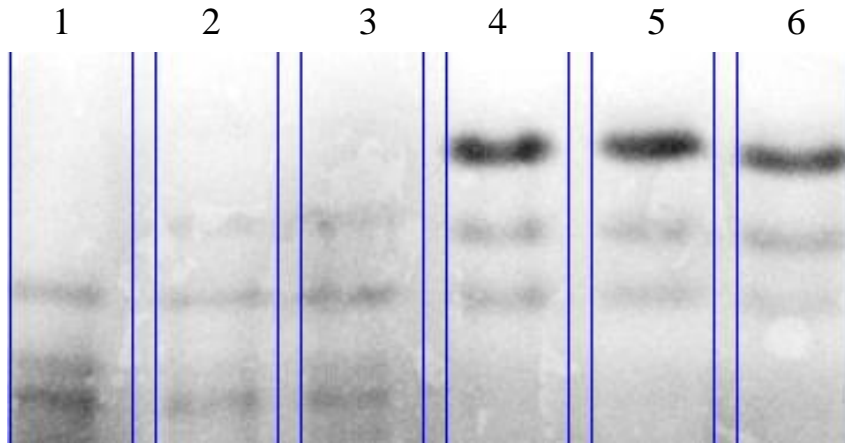
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Site	Name	Coordinates (Lambert 93)	Species	Waterbody	Characteristics
No1	Courcelles-lès-lens	x:701904.14 y:7036413.73	D. r. bug.	Leisure base on the Deûle waterway	Former harbour; located between two factories (Pb, Cd, Zn)
No2	Condé-sur-l'Escaut	x:742523.91 y:7040341.87	D. pol.	Pond	Former coal mining area
Oi1	Verneuil-en-Halatte	x:664328.03 y:6909078.27	D. pol., D. r. bug.	Canalized Oise River	Downstream a waste water treatment plant; commercial shipping
Mo1	Montigny - canal de Jouy	x:930770.97 y:6894478.49	D. pol., D. r. bug.	Canalized Moselle River	Two adjacent waterways located in the Metz agglomeration; wood treatment plant upstream using metal salts and creosote, and a former tar refinery, closed in 1996 (PAHs, phenols, naphthalene, creosote).
Mo2	Montigny - canal de Metz	x:931048.51 y:6894967.27	D. r. bug.	Derivation channel of the Moselle River	
Mo3	Jouy aux arches	x:924931.85 y:6889507.53	D. r. bug.	Canalized Moselle River	Upstream from Mo1 and Mo2
Mo4	Liverdun	x:925364.58 y:6853881.56	D. r. bug., D. pol.	Canalized Moselle River	Upstream from Mo1, Mo2 and Mo3; before the confluence with the Meurthe River
Me1	Saint-Mihiel	x:885822.17 y:6868219.32	D. pol., D. r. bug.	Lock on the Canal de l'Est branche Nord	Recreational activities (boating , shipping, fishing)
Me2	Madine	x:901250.30 y:6873460.86	D. pol.	Lake fed by a Tributary of Moselle River	Leisure base, prohibited to thermal motor boats; reserve of drinking water for the city of Metz
So1	Riaucourt	x:859800.49 y:6788737.27	D. pol.	Waterway (Canal entre Champagne et Bourgogne)	Used for commercial shipping, frequently emptied (fluctuating water level)
So2	Scey-sur-Saône	x:923259.17 y:6732308.04	D. pol.	Marina located on the Saône River	
So3	Seurre	x:862786.46 y:6657960.18	D. r. bug., D. pol.	Harbour between the Saone and its diversion channel	A boat sank on September 2015, leading to a release of fuel within the harbour
So4	Nantua	x:900751.79 y:6565109.42	D. pol.	Lake of glacial origin	Classified as a protected natural site since 1936

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784 *Figure A.1.: Cu/Zn-SOD patterns from D. polymorpha (lanes 1-3) and D. r. bugensis (lanes 4-6)*

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786 *Cu/Zn-SOD activities were determined after isoelectrofocusing in native conditions directly on gel by*
787 *photochemical revelation with nitroblue tetrazolium. The gels were scanned and subsequently*
788 *analyzed using the Image Lab 4.1 software developed by BIO-RAD. Observed patterns clearly*
789 *discriminate D. polymorpha and D. r. bugensis. In this example, individuals were collected at Saint-*
790 *Mihiel.*

791

792 Table A.2.: Mean \pm standard deviation for the 11 biomarkers considered (part 1)

		Site	ProtB mg/gFW	Tri mg/gFW	Chol mg/gFW	ETS $\mu\text{molO}_2\text{.gProt-1}$	LOOH $\mu\text{mol/gProt}$
October 2015	<i>D. r. bugensis</i>	No1	48,9 \pm 5,6	23,8 \pm 6,4	2,2 \pm 0,3	1 \pm 0,3	75 \pm 58,9
		Mo1	57,7 \pm 9,1	15,1 \pm 7,2	2,3 \pm 0,3	0,9 \pm 0,4	38,7 \pm 36,8
		Mo3	57,1 \pm 7,8	29 \pm 5,9	2,6 \pm 0,3	0,8 \pm 0,2	72,7 \pm 77
		Mo4	51,2 \pm 6,4	23,2 \pm 5,7	2,4 \pm 0,2	0,9 \pm 0,2	66,1 \pm 58,3
		Me1	50,4 \pm 7,2	20,9 \pm 7,4	2,3 \pm 0,4	0,9 \pm 0,3	60,3 \pm 65,8
		So3	49,4 \pm 5,4	15,4 \pm 5,8	2,3 \pm 0,3	0,9 \pm 0,2	36,8 \pm 35,2
	<i>D. polymorpha</i>	No2	52,1 \pm 8	16,8 \pm 3,8	1,9 \pm 0,2	1,4 \pm 0,3	38,9 \pm 36,7
		Oi1	52,5 \pm 8,2	1,6 \pm 0,9	1,9 \pm 0,3	1,6 \pm 0,3	16,9 \pm 3,7
		Mo1	52,8 \pm 10,9	2,4 \pm 2,1	1,7 \pm 0,2	1,4 \pm 0,4	18,5 \pm 10
		Me1	58,6 \pm 7,5	22,1 \pm 4,8	2,6 \pm 0,3	1,3 \pm 0,2	50,2 \pm 61,5
		Me2	58,5 \pm 6	2,7 \pm 1,9	2,1 \pm 0,2	1,4 \pm 0,3	25,8 \pm 29,1
		So1	51,4 \pm 7,9	2 \pm 0,9	2 \pm 0,4	1,4 \pm 0,5	23,4 \pm 18,6
		So2	54,2 \pm 7	3,5 \pm 3,2	2 \pm 0,3	1,3 \pm 0,4	26,9 \pm 32
		So4	54,1 \pm 6,8	7,8 \pm 3,9	2 \pm 0,3	1,9 \pm 0,6	17,6 \pm 4,8
April 2016	<i>D. r. bugensis</i>	No1	52,6 \pm 5,1	17,6 \pm 10,4	2,3 \pm 0,6	0,9 \pm 0,2	69,5 \pm 65,5
		Oi1	52,8 \pm 2,3	13,5 \pm 5	2,3 \pm 0,6	0,8 \pm 0,2	27,5 \pm 26,8
		Mo1	64,3 \pm 4,2	28 \pm 4,2	3,3 \pm 1	0,6 \pm 0,3	109,2 \pm 77,8
		Mo2	54,6 \pm 12,8	7,6 \pm 5,8	2,2 \pm 0,4	0,7 \pm 0,3	18,5 \pm 12,8
		Mo3	57,3 \pm 2,5	16,5 \pm 6,1	2,5 \pm 0,4	0,8 \pm 0,2	45,9 \pm 50,5
		Mo4	52,8 \pm 3,5	15 \pm 6,2	2,6 \pm 0,6	0,9 \pm 0,2	25,5 \pm 32,9
		Me1	56 \pm 4,7	24,8 \pm 3,5	2 \pm 0,7	0,7 \pm 0,1	125,2 \pm 77,5
		So3	54,7 \pm 2,6	18,9 \pm 4	2,5 \pm 0,6	0,7 \pm 0,2	47,6 \pm 48
	<i>D. polymorpha</i>	No2	64,2 \pm 16,2	18,2 \pm 3,9	3 \pm 1,8	1,2 \pm 0,2	40,6 \pm 16
		Oi1	64,6 \pm 3,2	4,8 \pm 2,2	2,1 \pm 0,4	1,2 \pm 0,1	20,4 \pm 21,1
		Mo1	57,4 \pm 2,9	15,5 \pm 5,2	1,9 \pm 0,4	1,2 \pm 0,2	39,5 \pm 59,9
		Me1	65,7 \pm 3	21,3 \pm 3,8	2,1 \pm 0,6	1,3 \pm 0,2	56,8 \pm 66,1
		Me2	62,9 \pm 4,9	18,9 \pm 4,6	2,3 \pm 0,3	1,1 \pm 0,2	41,2 \pm 62,3
		So1	54,4 \pm 6,1	10,3 \pm 6,5	2,1 \pm 0,5	1,1 \pm 0,2	55,1 \pm 75,2
February 2017	<i>D. r. bugensis</i>	No1	50,6 \pm 5,2	19,3 \pm 4,7	2,3 \pm 0,2	0,9 \pm 0,2	40,9 \pm 55,7
		Oi1	52,9 \pm 4,1	13,6 \pm 3,2	2 \pm 0,5	0,8 \pm 0,1	17,8 \pm 16,1
		Mo1	55,6 \pm 5,3	21,4 \pm 3,8	3,1 \pm 0,5	0,8 \pm 0,2	46,5 \pm 53,3
		Mo2	52,3 \pm 10,4	11,2 \pm 5,2	2,1 \pm 0,3	0,9 \pm 0,3	21,3 \pm 10,8
		Mo3	53,7 \pm 6,5	12,4 \pm 5,6	2,7 \pm 0,3	0,8 \pm 0,2	19,4 \pm 12
		Mo4	55 \pm 4,6	14,7 \pm 2,2	2,7 \pm 0,2	0,8 \pm 0,1	24,3 \pm 32,2
		So3	56,5 \pm 6,4	20,2 \pm 7,7	2,5 \pm 0,4	0,8 \pm 0,2	50,2 \pm 55,9
		<i>D. polymorpha</i>	No2	59,1 \pm 3,9	16 \pm 4	2,4 \pm 0,5	1,1 \pm 0,1
	Oi1		57,2 \pm 5,1	6,7 \pm 2,9	2,2 \pm 0,3	1,1 \pm 0,2	12,4 \pm 2,2
	Mo1		56,8 \pm 3,9	14,7 \pm 3,3	2,8 \pm 0,3	1 \pm 0,1	14,3 \pm 2
	Mo4		59,2 \pm 6,4	5,6 \pm 1,1	2 \pm 0,2	1,1 \pm 0,2	12 \pm 1,4
	Me1		61,3 \pm 4,5	8,8 \pm 2,5	2,3 \pm 0,3	1 \pm 0,1	11 \pm 2,8
	Me2		62,8 \pm 4,8	18,1 \pm 4	2,6 \pm 0,5	1 \pm 0,1	14 \pm 1,9
	<i>D. polymorpha</i>	So1	59,9 \pm 4,1	20 \pm 2,5	2,1 \pm 0,2	1,1 \pm 0,1	15,7 \pm 2,7
So2		61,9 \pm 6,6	11,3 \pm 3,8	2,4 \pm 0,5	1 \pm 0,1	15,4 \pm 15,3	
So3		61,4 \pm 6,7	12,1 \pm 5,4	2,4 \pm 0,3	1,1 \pm 0,2	13,6 \pm 2,2	
So4		54,5 \pm 5,5	13,6 \pm 3,8	2,1 \pm 0,2	1,2 \pm 0,2	12,9 \pm 2,6	

Table A.2.: Mean \pm standard deviation for the 11 biomarkers considered (part 2)

	Site	ACP	ProtFC	GST	TAC	LDH	Csp	
		$\mu\text{mol/gprot/h}$	mg/gFW	$\mu\text{molCDNB/gprot/r}$	$\mu\text{molTeq/gprot}$	$\mu\text{molNADH/gprot}$	$\mu\text{molpNA/gpro t/h}$	
October 2015	<i>D. r. bugensis</i>	No1	69,9 \pm 11,8	34,2 \pm 4,8	229,4 \pm 45,2	15,7 \pm 6,6	170,8 \pm 69,1	5,3 \pm 2,4
		Mo1	107,2 \pm 30,2	38,7 \pm 6,1	393 \pm 128,7	18,6 \pm 6,7	139,6 \pm 48,2	7,5 \pm 4,7
		Mo3	90,7 \pm 16,9	38 \pm 5,3	346 \pm 80,6	12,2 \pm 6,9	141,6 \pm 37,3	7,7 \pm 2
		Mo4	96,7 \pm 18	35,8 \pm 4,9	311,7 \pm 67,2	14,2 \pm 7,8	165,3 \pm 53,9	7,4 \pm 3
		Me1	109,7 \pm 21,8	33,7 \pm 5,5	319 \pm 76,5	17,4 \pm 6,5	182,4 \pm 59,7	13,2 \pm 9,6
		So3	95 \pm 31,2	34,3 \pm 4,4	268,7 \pm 42,1	10,3 \pm 4,7	144,9 \pm 23	3,5 \pm 0,9
	<i>D. polymorpha</i>	No2	78,4 \pm 18,5	39 \pm 5,5	246,2 \pm 107,9	23,9 \pm 6,3	121,5 \pm 29,8	8,7 \pm 2,1
		Oi1	97,7 \pm 22,2	35,4 \pm 6,1	539,7 \pm 139,4	28 \pm 8,4	194,5 \pm 98,5	17,8 \pm 12,5
		Mo1	74,7 \pm 15,1	35,2 \pm 7,5	497,1 \pm 200,1	31,8 \pm 5,9	185,5 \pm 54,5	10,8 \pm 6,7
		Me1	94 \pm 22,3	41 \pm 5	300,2 \pm 74	20,5 \pm 5,5	137,8 \pm 36,8	18,8 \pm 6,5
		Me2	58,9 \pm 13,6	36,8 \pm 4,1	518,3 \pm 179,4	26,1 \pm 4,2	140,1 \pm 38,3	20,3 \pm 5,6
		So1	84,4 \pm 29,6	34,7 \pm 4,6	384,2 \pm 111,8	23,9 \pm 6,5	109,5 \pm 38,9	4,6 \pm 2,8
		So2	82,3 \pm 20,8	37,1 \pm 5,1	349,8 \pm 98,3	24,5 \pm 3,5	116,1 \pm 21,6	6,2 \pm 3
		So4	100 \pm 18,2	36,2 \pm 5,7	556,8 \pm 287,7	37,1 \pm 3,8	170,6 \pm 61,4	7,2 \pm 4,5
April 2016	<i>D. r. bugensis</i>	No1	71,5 \pm 27,3	53,5 \pm 58,6	163,8 \pm 56,5	13,5 \pm 3,6	167 \pm 64,7	3,5 \pm 1,9
		Oi1	90,2 \pm 10,2	41,9 \pm 3,9	172,6 \pm 27,4	14,1 \pm 3,1	169,6 \pm 28,2	4,2 \pm 1,3
		Mo1	99,8 \pm 29,5	42,8 \pm 4,1	192 \pm 31,7	13,3 \pm 5,6	152,1 \pm 44,6	6,6 \pm 2,4
		Mo2	60,7 \pm 20,7	35,8 \pm 4,7	209,8 \pm 68,1	15,6 \pm 3,4	164,3 \pm 51,6	4 \pm 1,1
		Mo3	87,1 \pm 19,3	41,8 \pm 4,5	199,6 \pm 46,6	16,1 \pm 2,9	203,1 \pm 31,1	5,8 \pm 1,9
		Mo4	78,2 \pm 15,8	36,1 \pm 7	214,8 \pm 48,8	17,1 \pm 7,1	227,1 \pm 73,7	6,7 \pm 2,6
		Me1	63,6 \pm 13	36 \pm 3,6	218,5 \pm 30,2	15,4 \pm 4,9	171,5 \pm 39	4,4 \pm 1,2
		So3	98,4 \pm 39,3	39,7 \pm 4,1	219,7 \pm 53,4	15,1 \pm 5,4	198,3 \pm 60,5	4,4 \pm 1,4
	<i>D. polymorpha</i>	No2	57,3 \pm 8,9	49,4 \pm 16,7	192 \pm 52	26,6 \pm 2,8	127,3 \pm 19,5	8 \pm 2,5
		Oi1	54,5 \pm 10,4	44 \pm 2,7	293,3 \pm 92,6	27,8 \pm 2,8	150,2 \pm 20,9	11,4 \pm 5,5
		Mo1	59,1 \pm 16,9	43,5 \pm 3,9	260,3 \pm 90,7	28 \pm 5,7	143 \pm 34,6	7 \pm 1,9
		Me1	64,9 \pm 15,2	48,1 \pm 3,5	199,5 \pm 71,3	21,2 \pm 5,4	120,9 \pm 25,2	10,9 \pm 4,2
		Me2	56,8 \pm 8,8	43 \pm 6,1	245,9 \pm 57,9	26,5 \pm 4,3	166,5 \pm 22,1	7,3 \pm 2,3
		So1	39,4 \pm 9,6	37 \pm 6,8	252,7 \pm 108,9	24,4 \pm 6,9	143,2 \pm 46,5	4,6 \pm 3,3
February 2017	<i>D. r. bugensis</i>	No1	80,6 \pm 14,1	37,2 \pm 3,4	198,8 \pm 37	15,7 \pm 3,2	182,3 \pm 31	3,2 \pm 1,9
		Oi1	122 \pm 24,9	39,4 \pm 3,6	271,8 \pm 34	15,9 \pm 3,5	190,3 \pm 31,3	3,6 \pm 1,5
		Mo1	127,5 \pm 17,7	39,4 \pm 3,6	416,2 \pm 53,2	17,8 \pm 3,2	147,5 \pm 48,7	1,9 \pm 1,9
		Mo2	99,9 \pm 30,9	36,5 \pm 3,5	323,6 \pm 61,3	15,9 \pm 3,1	166,6 \pm 49,2	3,5 \pm 2,9
		Mo3	109,3 \pm 21,7	39,7 \pm 2,9	313,3 \pm 63,9	17 \pm 4,1	191,3 \pm 39,3	4,1 \pm 2,3
		Mo4	106,8 \pm 18,4	40,3 \pm 2,4	283,3 \pm 55,2	14,2 \pm 4	178,1 \pm 39,3	3,1 \pm 1,8
	<i>D. polymorpha</i>	So3	123 \pm 33	40,9 \pm 2,8	256,2 \pm 50,3	13,5 \pm 3,8	143,6 \pm 44,2	2,2 \pm 1,5
		No2	72,2 \pm 14,8	44,4 \pm 3,4	291,9 \pm 70,4	23,8 \pm 5	124,7 \pm 20	4 \pm 4,1
		Oi1	111,2 \pm 25,5	40,9 \pm 3,1	524 \pm 141,2	27,6 \pm 6,6	148,2 \pm 20,1	6,8 \pm 8,3
		Mo1	88,3 \pm 13,2	40,9 \pm 3,2	379,8 \pm 89,1	27,2 \pm 4,3	121 \pm 36,5	3,8 \pm 2,9
		Mo4	84,1 \pm 22,6	42,9 \pm 3,8	294,6 \pm 80,8	28,6 \pm 6,8	148,5 \pm 20,2	8,3 \pm 4,8
		Me1	60,9 \pm 9,4	44,8 \pm 2,9	283,9 \pm 71,4	21,1 \pm 4,7	114,6 \pm 18,2	6,9 \pm 6
		Me2	77,9 \pm 15,8	45,5 \pm 3,8	360,1 \pm 56,9	21,1 \pm 6,5	138,3 \pm 24,2	2,4 \pm 2,1
		So1	75,4 \pm 11,2	45,9 \pm 2,7	289,9 \pm 41,3	29,1 \pm 4,8	140,4 \pm 7,2	3,5 \pm 1,6
<i>D. polymorpha</i>	So2	72,2 \pm 16,1	45,6 \pm 2,3	231,4 \pm 73	24,9 \pm 5	121,6 \pm 27,6	3,4 \pm 3,9	
	So3	91 \pm 20,3	42,8 \pm 4	426,9 \pm 117,8	26,4 \pm 7,9	140,9 \pm 29,4	6,3 \pm 8,6	
	So4	76,1 \pm 10,1	41,7 \pm 2,4	358,9 \pm 69,4	30 \pm 6,3	150,4 \pm 19,7	3,1 \pm 2	

October 2015

site	No1	No2	Oi1	Mo1	Mo3	Mo4	Me1	Me2	So1	So2	So3	So4
Anthracene	1304	334	122	841	114	375	48	42	390	10	27	513
Benzo(a)anthracene	5007	1108	218	3324	494	1435	214	487	2890	46	190	2362
Benzo(a)pyrene	5291	920	203	3380	503	1265	82	425	2600	52	248	2253
Benzo(ghi)perylene	2770	609	133	1785	306	976	210	239	1570	48	289	1280
Benzo(k)fluoranthene	3073	564	127	1920	232	828	163	198	1490	27	140	1211
Chrysene	5310	1102	204	3687	492	1496	230	482	2560	25	217	2293
Fluoranthene	12072	2065	448	7762	1142	3306	737	940	5909	90	509	4586
Indeno(1)pyrene	1607	334	70	1140	172	535	130	150	955	14	79	791
Naphtalene	130	182	11	1043	5	70	18	5	81	5	5	5
PCB101	2,5	9	2,5	2,5	2,5	34	2,5	2,5	2,5	2,5	2,5	2,5
PCB118	2,5	12	2,5	2,5	2,5	13	2,5	2,5	2,5	2,5	2,5	2,5
PCB138	2,5	2,5	2,5	2,5	2,5	22	2,5	2,5	2,5	2,5	2,5	2,5
PCB153	2,5	2,5	2,5	2,5	2,5	22	2,5	2,5	2,5	2,5	2,5	2,5
PCB180	2,5	2,5	2,5	2,5	2,5	8	2,5	2,5	2,5	2,5	2,5	2,5
PCB28	2,5	7	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
PCB52	2,5	11	2,5	2,5	2,5	13	2,5	2,5	2,5	2,5	2,5	2,5
Phenanthrene	4579	1620	148	2606	489	1611	203	223	1910	25	191	1687
Sum PCB	2,5	44	2,5	2,5	2,5	112	2,5	2,5	2,5	2,5	2,5	2,5
Al	23070	84850	15920	40840	65680	57310	29580	8708	25840	49250	43790	4338
As	16,2	8,2	3,6	11,4	26,4	30,6	7,8	21,9	22,5	24	15,4	4,5
Cd	54,7	0,4	0,1	0,3	1	0,9	0,3	0,2	0,3	0,2	0,8	0,2
Cr	62,4	122	46,5	81,5	93,3	80,8	53,9	23	116,8	58,3	59,7	42,2
Co	6,1	17,9	3,9	6,8	13,3	10	7,2	5,4	7,7	8	8,1	2,5
Cu	79	58,3	12,1	44,9	44,1	29,6	23,1	5,9	16,5	13	108,5	39,8
Fe	14740	25250	11150	27660	40480	41090	17230	24230	42200	19130	22900	6848
Mn	346,3	695,8	258,2	882,3	692,9	470,1	204,2	336,5	235,4	188,1	372,9	126,4
Hg	4,46	0,16	0,07	0,04	0,2	0,28	0,06	0,02	0,05	0,01	0,14	0,52
Ni	17,6	49	8,6	16	42	24,1	22,6	10,6	28	16	23,6	21,7
Pb	1004	30,4	15,7	69,7	61,8	355,4	18,6	8,5	25,2	24,3	34,8	143,5
Zn	2443	192,8	60,2	109,9	275,9	853,9	122,5	41,3	129,1	48,3	265	128

February 2017

site	No1	No2	Oi1	Mo1	Mo2	Mo3	Mo4	Me1	Me2	So1	So2	So3	So4
Anthracene	1601	298	77	104	136	2114	39	119	10	884	76	91	138
Benzo(a)anthracene	2593	661	142	249	337	2260	99	339	34	1737	153	263	412
Benzo(a)pyrene	2451	643	173	315	339	2591	120	407	32	1900	200	324	430
Benzo(ghi)perylene	1340	460	125	210	213	1372	76	273	35	1092	129	207	265
Benzo(k)fluoranthene	1257	319	87	146	164	1172	55	197	18	882	99	152	216
Chrysene	3367	719	160	305	413	3154	115	382	25	2128	189	304	434
Fluoranthene	5429	1413	286	526	630	6761	197	678	74	3914	326	499	666
Indeno(1)pyrene	945	383	101	172	169	1210	62	227	21	944	104	167	226
Naphtalene	145	306	13	12	31	137	5	26	5	69	36	12	50
PCB101	2,5	27	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
PCB118	2,5	28	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
PCB138	8	19	2,5	2,5	2,5	5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
PCB153	8	17	2,5	2,5	2,5	7	2,5	2,5	2,5	2,5	2,5	2,5	2,5
PCB180	2,5	8	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
PCB28	2,5	34	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
PCB52	2,5	43	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
Phenanthrene	1583	1105	104	248	351	2878	70	288	25	1730	126	178	234
Sum PCB	22	176	2,5	2,5	2,5	12	2,5	2,5	2,5	2,5	2,5	2,5	2,5
Al	24180	25640	20500	36600	36400	78690	63480	41710	15160	20360	68450	40210	3268
As	19,2	7,1	5,7	8,4	10,9	24,1	14,7	8,2	10,1	29,2	32,4	18	2,6
Cd	59,5	0,5	0,3	0,1	0,1	1	0,1	0,4	0,1	0,1	0,8	0,5	0,1
Cr	69,3	146,2	62,6	84,5	113,6	85,4	60,1	50,1	41,6	121,7	89,8	61	14,9
Co	6,7	16,5	5,4	3,1	3,9	11,1	3,9	7,1	2,8	7,6	15,9	10,3	1
Cu	80,7	64,4	13,9	14,4	13,4	49,2	10	37,9	8	13,8	88,5	22,3	19,1
Fe	17160	20860	14310	18350	16720	37710	12760	22880	10030	50980	40280	23780	4869
Mn	319,6	1963	338,7	483,1	259,6	524,8	123,8	208	257,8	236	588,5	507,6	96,5
Hg	4,14	0,07	0,08	0,01	0,08	0,18	0,04	0,07	0,01	0,05	0,2	0,27	0,1
Ni	19,2	38,8	12,9	9	12,7	34,8	10	23,4	11,5	29,2	43,4	23,4	5
Pb	982,3	45,8	17,7	33,9	27,2	66,9	42,9	26,1	13,5	30,9	62,7	25,1	32,2
Zn	2851	182,9	71,7	35,6	62,5	244,8	76,9	148,6	30,3	130,9	432,3	164,3	92,5

799 Table A.4.: Internal concentrations measured on both species

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		<i>D. r. bugensis</i>						
	site	No1	Oi1	Mo1	Mo2	Mo3	Mo4	So3
Anthracene	µg/kg MS	102.983	38.869	22.547	23.764	28.437	38.047	24.343
Benzo(a)anthracene	µg/kg MS	88.26	57.149	29.874	53.651	72.362	31.639	25.31
Benzo(a)pyrene	µg/kg MS	42.032	24.632	13.478	34.013	37.285	19.666	20.349
Benzo(ghi)perylene	µg/kg MS	22.342	13.37	5.964	16.449	21.27	14.011	5.288
Benzo(k)fluoranthene	µg/kg MS	91.384	35.509	38.467	87.994	77.362	16.854	26.086
Chrysene	µg/kg MS	95.269	63.602	43.753	62.578	70.67	47.006	45.011
Fluoranthene	µg/kg MS	166.87	46.101	128.249	135.833	142.681	89.977	88.758
Indeno(1)pyrene	µg/kg MS	15.299	8.088	6.255	9.615	18.178	12.233	5.69
Phenanthrene	µg/kg MS	51.019	41.828	86.398	80.856	88.24	43.737	58.342
PCB101	µg/kg MS	69.283	25.056	13.958	22.687	19.49	22.371	24.397
PCB118	µg/kg MS	37.122	14.46	7.916	11.155	11.673	14.327	11.704
PCB138	µg/kg MS	77.594	26.762	16.313	27.662	27.106	35.041	33.377
PCB153	µg/kg MS	153.962	57.559	35.09	71.741	65.502	81.736	70.433
PCB180	µg/kg MS	117.951	<5.19	<4.89	<5.46	34.229	<9.43	<4.99
PCB28	µg/kg MS	<5.18	<5.19	<4.89	<5.46	<6.98	<9.43	<4.99
PCB52	µg/kg MS	23.39	11.617	9.389	9.667	8.238	<9.43	6.85
Al	mg/kg MS	379.7	639.6	95.38	774.2	1097	520.3	1250
As	mg/kg MS	4.99	3.54	5.25	6.32	7.57	5.71	5.21
Cd	mg/kg MS	8.84	0.657	0.561	1.12	1.2	0.648	0.47
Cr	mg/kg MS	1.8	2.4	0.423	1.92	2.79	1.5	2.55
Co	mg/kg MS	1	1.46	0.389	1.52	1.98	0.697	1.01
Cu	mg/kg MS	12.73	12.4	16.7	13.3	14.09	12.76	12.07
Fe	mg/kg MS	991	1840	342.4	1801	2135	1687	1744
Mn	mg/kg MS	94.53	213.6	36.22	498.3	638.3	78.34	81.16
Hg	mg/kg MS	0.369	0.115	0.074	0.163	0.21	0.148	0.18
Ni	mg/kg MS	4.45	5.92	1.95	6.15	5.93	3.39	4.36
Pb	mg/kg MS	86.51	2.29	0.853	2.62	4.04	1.15	1.95
Zn	mg/kg MS	397.8	102.3	76.34	92.18	91.97	76.16	86.09

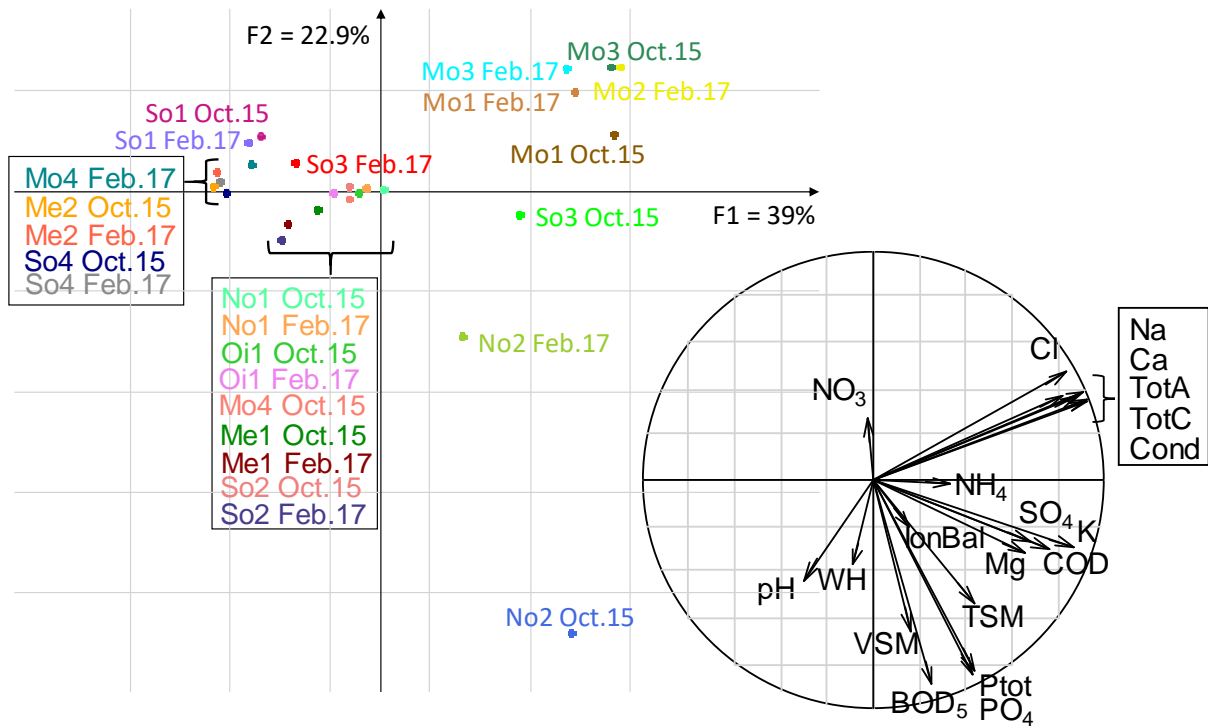
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		<i>D. polymorpha</i>							
	site	No2	Oi1	Me1	Me2	Mo1	So1	So2	So4
Anthracene	µg/kg MS	27.222	<17.61	6.235	<18.66	4.736	29.357	18.722	8.104
Benzo(a)anthracene	µg/kg MS	25.488	37.865	16.624	<18.66	8.393	23.234	14.089	15.121
Benzo(a)pyrene	µg/kg MS	8.403	20.244	8.117	22.207	6.874	13.201	12.328	12.966
Benzo(ghi)perylene	µg/kg MS	<2.49	<17.61	5.778	<18.66	<2.58	6.838	3.891	16.7
Benzo(k)fluoranthene	µg/kg MS	8.096	20.73	5.363	<18.66	23.809	15.497	13.849	14.622
Chrysene	µg/kg MS	27.155	63.461	15.824	31.737	21.275	41.564	25.192	31.542
Fluoranthene	µg/kg MS	84.845	56.426	37.23	99.017	45.757	64.556	74.732	64.879
Indeno(1)pyrene	µg/kg MS	<2.49	<17.61	5.458	<18.66	<2.58	4.971	<2.47	4.806
Phenanthrene	µg/kg MS	51.228	46.101	16.033	92.644	31.712	24.025	128.73	35.903
PCB101	µg/kg MS	34.7	<35.21	<4.88	<37.31	<5.15	6.017	8.147	<5.1
PCB118	µg/kg MS	8.527	<35.21	<4.88	<37.31	<5.15	<5.0	<4.93	<5.1
PCB138	µg/kg MS	24.57	<35.21	<4.88	<37.31	<5.15	8.123	9.757	<5.1
PCB153	µg/kg MS	43.616	48.915	<4.88	<37.31	10.244	12.418	18.003	5.339
PCB180	µg/kg MS	<4.98	<35.21	<4.88	<37.31	<5.15	<5.0	5.264	<5.1
PCB28	µg/kg MS	<4.98	<35.21	<4.88	<37.31	<5.15	<5.0	<4.93	<5.1
PCB52	µg/kg MS	27.404	<35.21	<4.88	<37.31	<5.15	<5.0	<4.93	<5.1
Al	mg/kg MS	19.75	568.9	145.6	261.4	115.1	248.8	308.6	67.35
As	mg/kg MS	2.75	3.79	4.24	4.26	5.21	4.41	5.77	4.37
Cd	mg/kg MS	0.134	1.07	0.451	0.402	0.659	0.445	0.187	0.478
Cr	mg/kg MS	0.162	3.04	0.464	0.775	0.435	2.81	0.827	0.87
Co	mg/kg MS	0.656	1.65	0.598	0.686	0.641	0.67	0.766	0.533
Cu	mg/kg MS	10.67	15.21	16.68	11.77	11.84	14.49	17.26	13.14
Fe	mg/kg MS	190.3	1618	274.5	540.3	267.2	568.2	513.3	399.5
Mn	mg/kg MS	85.55	138.7	14.32	23.31	46.84	18	32.59	16.76
Hg	mg/kg MS	<0.025	0.114	0.032	0.06	0.112	0.053	0.03	0.056
Ni	mg/kg MS	6.01	10.83	4.65	3.83	3.42	12.25	3.4	3.57
Pb	mg/kg MS	0.216	1.68	0.281	0.354	0.603	0.418	0.518	0.372
Zn	mg/kg MS	85.64	98.81	79.84	75.25	74.46	85.96	93.84	93.45

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806 *Figure A.2.: PCA results from the water physico-chemistry measured for 12 sites x 2 seasons (October*
 807 *2015: Oct.15, February 2017: Feb.17), except for the Mo2 site analysed only in February 2017.*

808 Table A.5.: Results of PLS regressions on the relationship between biomarkers and sediment
 809 contamination (a); biomarkers and soft tissue contaminant accumulation (b); sediment contamination
 810 and bioaccumulation (c).

a	SEDIMENTS CONTAMINATION EXPLAINED BY BIOMARKERS			
	<i>D. r. bugensis</i>		<i>D. polymorpha</i>	
	October 2015	February 2017	October 2015	February 2017
	Proportion of variance explained cumulated on 3 axes (%)			
Input	72.43	78.91	87.37	76.21
Target	67.40	60.19	50.56	38.46
	Cumulative R ² on 3 axes - Input			
ProtB	0.95	0.96	0.85	0.58
Tri	0.83	0.97	0.97	0.95
Chol	1.00	0.70	0.90	0.74
ETS	1.00	0.91	0.84	0.54
LOOH	0.72	0.99	0.99	0.69
ACP	0.94	0.80	0.62	0.65
ProtFC	0.99	0.77	0.95	0.82
GST	0.89	0.35	0.99	0.80
TAC	0.43	0.29	0.87	0.86
LDH	0.65	0.91	0.73	0.72
Csp	0.24	0.96	0.80	0.90
Total explained	0.72	0.79	0.87	0.76
	Cumulative R ² on 3 axes - Target			
Anthracene	0.97	0.81	0.50	0.63
Benzo(a)anthracene	0.97	0.87	0.44	0.63
Benzo(a)pyrene	0.99	0.83	0.46	0.61
Benzo(ghi)perylene	0.97	0.84	0.42	0.59
Benzo(k)fluoranthene	0.98	0.86	0.42	0.61
Chrysene	0.98	0.86	0.42	0.63
Fluoranthene	0.97	0.95	0.39	0.62
Indenolpyrene	0.96	0.79	0.40	0.59
Naphtalene	0.99	0.86	0.16	0.15
PCB 101	0.06	0.00	0.53	0.07
PCB 118	0.06	0.00	0.53	0.07
PCB 138	0.06	0.93	0.00	0.07
PCB 153	0.06	0.90	0.00	0.07
PCB 180	0.06	0.00	0.00	0.07
PCB 28	0.00	0.00	0.53	0.07
PCB 52	0.06	0.00	0.53	0.07
Phenanthrene	0.96	0.72	0.51	0.56
Sum PCB	0.06	0.95	0.53	0.07
Al	0.84	0.44	0.77	0.70
As	0.60	0.26	0.80	0.64
Cd	0.97	0.93	0.67	0.38
Cr	0.91	0.20	0.82	0.30
Co	0.99	0.18	0.79	0.44
Cu	0.16	0.92	0.65	0.45
Fe	0.71	0.37	0.70	0.61
Mn	0.99	0.43	0.18	0.10
Hg	0.98	0.94	0.72	0.16
Ni	0.96	0.32	0.82	0.63
Pb	0.97	0.93	0.70	0.44
Zn	0.99	0.94	0.74	0.52
Total explained	0.67	0.60	0.51	0.38
	VIP on 3 axes			
ProtB	1.11	1.18	1.22	0.72
Tri	0.89	0.84	1.14	1.44
Chol	1.20	0.72	1.05	0.81
ETS	1.32	1.34	0.88	0.48
LOOH	0.88	0.76	0.83	1.25
ACP	1.19	1.28	0.68	0.85
ProtFC	1.18	1.04	0.92	1.12
GST	0.97	0.91	1.07	1.12
TAC	1.06	0.48	1.05	0.83
LDH	0.86	0.95	0.81	0.90
Csp	0.60	1.03	1.11	0.94

b	BIOACCUMULATION EXPLAINED BY BIOMARKERS	
	February 2017	
	<i>D. r. bugensis</i>	<i>D. polymorpha</i>
	Proportion of variance explained cumulated on 3 axes (%)	
Input	84.42	78.00
Target	74.75	54.57
	Cumulative R ² on 3 axes - Input	
ProtB	0.97	0.89
Tri	0.96	0.65
Chol	0.71	0.50
ETS	0.94	0.94
LOOH	0.99	0.60
ACP	0.82	0.87
ProtFC	0.74	0.84
GST	0.95	0.93
TAC	0.95	0.85
LDH	0.71	0.83
Csp	0.92	0.51
Total explained	0.84	0.78
	Cumulative R ² on 3 axes - Target	
Bioacc-Anthracene	0.83	0.60
Bioacc-Benzo(a)anthracene	0.87	0.46
Bioacc-Benzo(a)pyrene	0.85	0.76
Bioacc-Benzo(ghi)perylene	0.84	0.33
Bioacc-Benzo(k)fluoranthene	0.83	0.40
Bioacc-Chrysene	0.93	0.95
Bioacc-Fluoranthene	0.65	0.50
Bioacc-Indenolpyrene	0.44	0.56
Bioacc-Phenanthrene	0.62	0.31
Bioacc-PCB 101	0.96	0.13
Bioacc-PCB 118	0.90	0.74
Bioacc-PCB 138	0.93	0.32
Bioacc-PCB 153	0.92	0.11
Bioacc-PCB 180	0.87	0.85
Bioacc-PCB 28	0.32	0.85
Bioacc-PCB 52	0.91	0.19
Bioacc-Al	0.33	0.75
Bioacc-As	0.15	0.05
Bioacc-Cd	0.95	0.89
Bioacc-Cr	0.47	0.77
Bioacc-Co	0.57	0.83
Bioacc-Cu	0.97	0.02
Bioacc-Fe	0.78	0.89
Bioacc-Mn	0.61	0.55
Bioacc-Hg	0.86	0.63
Bioacc-Ni	0.68	0.54
Bioacc-Pb	0.94	0.88
Bioacc-Zn	0.95	0.44
Total explained	0.75	0.55
	VIP on 3 axes	
ProtB	1.16	1.05
Tri	1.00	0.76
Chol	0.83	0.83
ETS	1.21	1.12
LOOH	0.90	0.66
ACP	1.22	1.36
ProtFC	0.92	0.99
GST	1.21	1.30
TAC	0.89	0.96
LDH	0.85	1.08
Csp	1.00	0.75

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SEDIMENTS CONTAMINATIONS EXPLAINED BY BIOACCUMULATION

C	February 2017		February 2017		
	<i>D. r. bugensis</i>	<i>D. polymorpha</i>	<i>D. r. bugensis</i>	<i>D. polymorpha</i>	
	Proportion of variance explained cumulated on 3 axes (%)		Cumulative R ² on 3 axes - Target		
Input	88.04	72.04			
Target	62.95	68.34			
	Cumulative R ² on 3 axes - Input				
Bioacc-Anthracene	0.98	0.86	Anthracene	0.80	0.75
Bioacc-Benzo(a)anthracene	0.87	0.81	Benzo(a)anthracene	0.88	0.75
Bioacc-Benzo(a)pyrene	0.98	0.67	Benzo(a)pyrene	0.84	0.75
Bioacc-Benzo(ghi)perylene	0.84	0.28	Benzo(ghi)perylene	0.84	0.77
Bioacc-Benzo(k)fluoranthene	0.84	0.33	Benzo(k)fluoranthene	0.86	0.76
Bioacc-Chrysene	0.94	0.94	Chrysene	0.87	0.75
Bioacc-Fluoranthene	0.92	0.47	Fluoranthene	0.98	0.75
Bioacc-Indenolpyrene	0.72	0.69	Indenolpyrene	0.81	0.77
Bioacc-Phenanthrene	0.94	0.16	PCB 101	0.00	0.92
Bioacc-PCB 101	0.99	0.98	PCB 118	0.00	0.92
Bioacc-PCB 118	0.99	0.95	PCB 138	0.99	0.92
Bioacc-PCB 138	0.95	0.93	PCB 153	0.86	0.92
Bioacc-PCB 153	0.92	0.91	PCB 180	0.00	0.92
Bioacc-PCB 180	0.97	0.85	PCB 28	0.00	0.92
Bioacc-PCB 28	0.10	0.91	PCB 52	0.00	0.92
Bioacc-PCB 52	0.89	1.00	Phenanthrene	0.80	0.81
Bioacc-Al	0.69	0.69	Al	0.49	0.25
Bioacc-As	0.79	0.67	As	0.46	0.36
Bioacc-Cd	1.00	0.82	Cd	0.99	0.20
Bioacc-Cr	0.87	0.97	Cr	0.55	0.75
Bioacc-Co	0.90	0.75	Co	0.36	0.61
Bioacc-Cu	0.93	0.43	Cu	0.88	0.46
Bioacc-Fe	0.99	0.88	Fe	0.62	0.65
Bioacc-Mn	0.94	0.61	Mn	0.21	0.88
Bioacc-Hg	0.91	0.64	Hg	0.98	0.15
Bioacc-Ni	0.82	0.96	Ni	0.57	0.62
Bioacc-Pb	0.99	0.74	Pb	0.99	0.57
Bioacc-Zn	0.99	0.31	Zn	0.99	0.33
Total explained	0.88	0.72	Total explained	0.63	0.68
	VIP on 3 axes				
Bioacc-Anthracene	1.09	1.76			
Bioacc-Benzo(a)anthracene	1.08	0.77			
Bioacc-Benzo(a)pyrene	1.08	0.85			
Bioacc-Benzo(ghi)perylene	0.99	0.96			
Bioacc-Benzo(k)fluoranthene	0.93	0.64			
Bioacc-Chrysene	1.10	0.77			
Bioacc-Fluoranthene	1.04	0.62			
Bioacc-Indenolpyrene	1.17	1.12			
Bioacc-Phenanthrene	0.95	0.60			
Bioacc-PCB 101	1.09	1.30			
Bioacc-PCB 118	1.10	0.98			
Bioacc-PCB 138	1.07	1.08			
Bioacc-PCB 153	1.05	1.41			
Bioacc-PCB 180	1.20	1.00			
Bioacc-PCB 28	0.33	1.06			
Bioacc-PCB 52	1.05	1.23			
Bioacc-Al	0.81	0.70			
Bioacc-As	1.10	1.12			
Bioacc-Cd	1.13	1.09			
Bioacc-Cr	0.77	1.24			
Bioacc-Co	0.89	0.46			
Bioacc-Cu	0.71	0.94			
Bioacc-Fe	0.76	0.68			
Bioacc-Mn	0.96	0.71			
Bioacc-Hg	1.15	1.11			
Bioacc-Ni	0.64	1.40			
Bioacc-Pb	1.12	0.63			
Bioacc-Zn	1.12	0.32			