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1 **Preliminary inter-port study of the quality of environments using physiological responses**
2 **of invertebrates exposed to chronic trace element and organic contamination in Corsica**
3 **(Mediterranean Sea)**

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30

31 **Abstract**

32 Port areas are socio-ecosystems impacted by chronic mixture pollution. Some marine species
33 benefit from living there and may be studied to define the ecological state of such environments.
34 In this study, the risks of chronic chemical contamination and its consequences on three marine
35 molluscs were evaluated in North Corsica (France) port areas. Mediterranean mussel *Mytilus*
36 *galloprovincialis*, tubular sea cucumber *Holothuria tubulosa* and Mediterranean limpet *Patella*
37 *sp.* were sampled in three port areas and a reference location. A set of biomarkers was analysed
38 to evaluate oxidative stress, detoxification, energetic metabolism, neurotoxicity, immunity and
39 bioaccumulation (metallic trace elements and organic pollutants). The objectives were to assess
40 pollution-induced effects in organisms, to determine the best bioindicator species for the
41 selected locations and to validate a “pool” sampling technique (when the analysis is done on a
42 single pool of samples and not on individual samples). The results validate the sampling
43 techniques as “pool” for management purposes. St-Florent was demonstrated as the most
44 contaminated location. All the other locations present a low contamination, below the
45 recommended threshold values (for metallic trace elements and organic pollutants). Finally, the
46 limpet appears to be the best bioindicator for the selected locations. Mussel and sea cucumber
47 are inappropriate due to their absence in this oligotrophic region and the lack of responses
48 observed, respectively.

49

50 Keywords: pollution mixture, bioindicators, multibiomarkers approach, trace element, organic
51 pollutants, IBR.

52

53 **Introduction**

54 Human activities at sea (e.g. fisheries and shipping), on land (e.g. agriculture and industries) or
55 along the coastline (e.g. wastewater treatment plant and massive tourism) cause chronic and
56 diffuse pollution in the marine environment (Islam and Tanaka 2004). Urban ports are thus
57 subjected to a myriad of pollutants coming from terrestrial discharges or anthropogenic
58 contaminations (Andral et al. 2004; Benali et al. 2015; Lafabrie et al. 2008). These pollutants
59 may represent a subsequent risk to human health (Fasulo et al. 2012; Maisano et al. 2017). In
60 these environments, biota is facing diverse stressful conditions and is exposed to a complex
61 mixture of organic and inorganic pollutants. Despite the growing interest in recreational boating
62 and the increasing number of marinas along coastlines all around the world, ecological
63 understanding of these ecosystems is still very scarce (Guerra-García et al. 2021).

64

65 In 2008, the Marine Strategy Framework Directive (DCSMM 2008) incited European states to
66 reduce the impact of activity in the marine environment, to achieve or maintain a good
67 environmental status. This status is one of the decisive factors for awarding eco-labels or eco-
68 certifications, such as Blue Flags in marinas (Guerra-García et al. 2021). It is thus crucial to
69 develop a strong method to assess and monitor contamination in the marine environment, and
70 also to understand the direct or combined effects of pollutants on organisms. Determining how
71 pollutants interact with other chemical stressors to cause additive or synergistic effects on
72 organisms remains one of the greatest challenges in ecotoxicology today. Also, direct chemical
73 analyses of water samples only give a snapshot of the presence of pollutants at a specific date
74 and time. Moreover, many environmental parameters (e.g., temperature, salinity, etc.) affect the

75 bioavailability of pollutants and their toxic effect on organisms could be modified.
76 Bioindicators are used to consider such influence. Bioindicators are sensitive organisms used
77 to observe induced effects of pollutants and therefore provide information on the impacts to the
78 environment. This evaluation can be measured at several levels, from the molecular and cellular
79 individual levels to the populations up to whole biocenosis level (Bondaruk et al., 2015). The
80 use of these bioindicators is particularly relevant from a biological point of view. The measured
81 concentrations reflect the bioavailable fractions in the marine environment (Wang 2016) and
82 confers an integrative measurement of their exposure and effect on ecologically available
83 fractions of environmental pollutants over time (Rainbow 2002).

84 Bivalves and more precisely mussels received the greatest attention (Luna-Acosta et al. 2015;
85 Rainbow and Phillips 1993) thanks to their biological characteristics (filter-feeders, global
86 distribution, large abundance, low mobility, easiness to sample, etc.). Mussels are routinely
87 used in regional environmental biomonitoring plans such as OSPAR's Coordinated
88 Environmental Monitoring Programme (CEMP), PNUMA's Program for the Assessment and
89 Control of Marine Pollution within the Mediterranean Action Plan (MED POL agreement of
90 the Barcelona Convention) or the Mussel Watch Project (USA), which was initiated in 1976
91 and includes the surveillance of coastal regions for their pollutant contents (Goldberg 1975).

92 However, other species (seagrass, fish, marine sponges, holothurians, limpets, etc.) are
93 sometimes better adapted to the environmental conditions of the studied environment and their
94 potential as bioindicators starts to increase (Beg et al. 2018; Lafabrie et al. 2007;
95 Mohammadizadeh et al. 2016; Orani et al. 2018; Parra-Luna et al. 2020; Pérez et al. 2019;
96 Richir and Gobert 2014). The use of native biota as a monitoring tool for bioavailable pollutants
97 in aquatic ecosystems was demonstrated to constitute a highly valuable approach (Bertrand et
98 al. 2018).

99 In the present study, a multibiomarkers approach was chosen to assess the impacts of multiple
100 contaminants. The term biomarker refers to “all the biochemical, cellular, physiological or
101 behavioural parameters which can be measured in the tissues or fluids of an organism or on the
102 whole organism, to highlight the exposure to, or the effects, one or more contaminants” (Garric
103 et al., 2010). Biomarkers were classified into two class: biomarkers of exposure indicate the
104 presence of a contaminant inside an organism due to its interaction with biological molecules,
105 and biomarkers of effect reflect the toxic or sublethal effect of the contaminant on the organism
106 (Luna-Acosta et al. 2017a). In this study, the bioaccumulation of multiple metallic trace
107 elements and organic pollutants represent the biomarkers of exposure. The activity of enzymes
108 involved in several metabolic processes were analysed as biomarkers of effect: antioxidant
109 defences (superoxide dismutase SOD - EC 1.15.1.1 and glutathione-S-transferase GST - EC
110 2.5.1.18), energetic pathways (lactate dehydrogenase LDH – EC 1.1.1.27, pyruvate kinase PK
111 – EC 2.7.1.40 and phosphoenolpyruvate carboxykinase PEPCK – EC 4.1.1.32), neurotoxicity
112 biomarker (acetylcholinesterase AChE - EC 3.1.1.7) and immune defence mechanisms (laccase
113 type phenoloxidase LAC - EC 1.10.3.2). This multibiomarkers approach was shown to be
114 efficient as it brings a better understanding and an integrated view of the biological responses
115 in environmental risk assessment (Cravo et al. 2009; Delahaut et al. 2019). However, this
116 approach needs to be coupled with an integrated tool such as the Integrated Biomarker Response
117 (IBR). The IBR summarizes biomarker responses and simplifies their interpretation in
118 biomonitoring programs (Beliaeff and Burgeot 2002). It was described to be useful for
119 evaluating the impact of environmental conditions on the health and physiological status of
120 organisms (Serafim et al. 2012). Compared to individual biomarkers, the IBR was useful to
121 distinguish locations along a stress gradient (Bertrand et al. 2018).

122 In the present study, different sampling techniques (with organisms collected as individual or
123 collected as a pool of individuals) were tested. The objective was to evaluate if analysing the

124 data as a pool, a less expensive and less time-consuming method, would give comparable results
125 to individual analyses. We hypothesised that this "pool" method could be proposed as a simple
126 and effective tool to local authorities for future environmental management.

127 The secondary objective was to select the best bioindicator for port areas, from several native
128 species. Two molluscs (*Mytilus galloprovincialis*, *Patella* sp.), and one echinoderm
129 (*Holothuria tubulosa*) were selected. *Mytilus galloprovincialis* was included to obtain a
130 reference species to compare with other studies while the other two species were available all
131 year long in all the sampling locations, easy to sample, and were identified as potential
132 bioindicator species in previous studies (Campanella et al. 2001; Mohammadizadeh et al. 2016;
133 Parra-Luna et al. 2020; Reguera et al. 2018). These three species allow a global view of the
134 different biological compartments since the mussel is a filter-feeder, the limpet is a grazer, and
135 the sea cucumber is a deposit-feeder. Our hypothesis was that both mussel and limpet would
136 give similar results while sea cucumber would be more contaminated due to its direct presence
137 on the sediment (where contaminants would accumulate).

138

139 Finally, the levels of contamination and the subsequent pollutant-induced stress syndrome were
140 compared in several North Corsica port areas, never studied before. We hypothesised that the
141 level of contamination would be quite low due to the restricted size of the considered port areas.

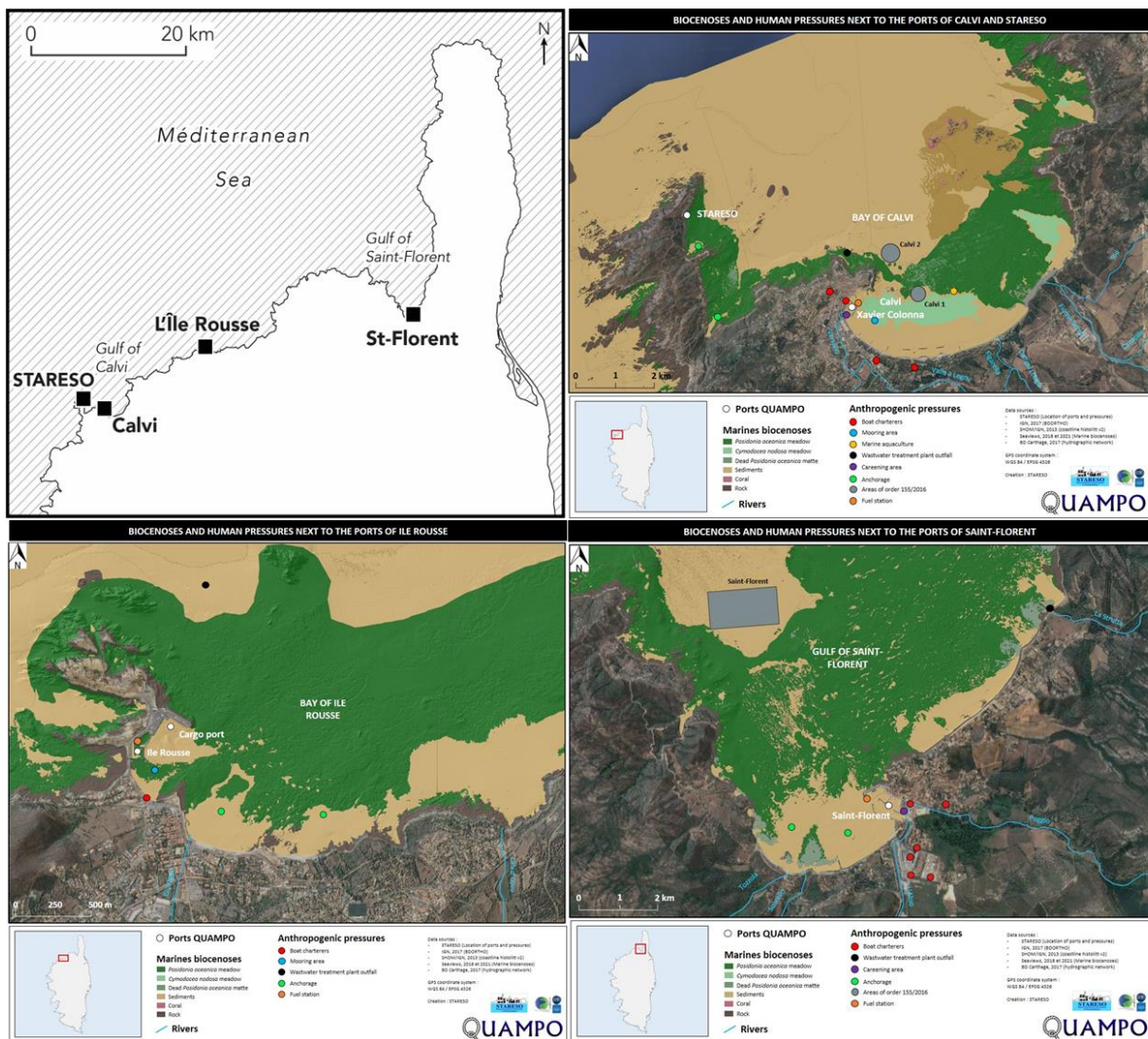
142

143 **Material and methods**

144 St-Florent (N 42° 40' 50.362"; E 9° 17' 54.373"), Île Rousse (N 42° 33' 59.116"; E 8° 56' 7.778")
145 and Calvi (N 42° 33' 59.116"; E 8° 45' 29.094") ports were chosen as exposed locations
146 (Figure 1). These three ports are small marinas with low hydrodynamism. St-Florent, Île-
147 Rousse and Calvi ports can respectively accommodate 950, 250 and 500 boats. All are located
148 close to boat rental agencies and oil stations; St-Florent and Calvi are also subjected to the

149 impact of their fairing area; and St-Florent is located at the mouth of the Aliso River. The
 150 STARESO station in the Revellata bay (N 42° 34' 49.584"; E 8° 43' 31.511") was chosen as the
 151 reference location (Figure 1). It is distant from any city and is used for only 2 boats. One water
 152 sample was taken from each location to evaluate the environmental trace element
 153 contamination.

154



155

156 **Fig. 1** Map of the experimental locations in North Corsica (France) and anthropogenic
 157 pressures observed next to the different ports
 158

159 Three species (*Mytilus galloprovincialis*, *Patella* sp. and *Holothuria tubulosa*) were sampled in
160 January 2020 in four different locations of North Corsica (France) (Figure 1). The objective
161 was to collect 20 mussel and limpet individuals (10 for bioaccumulation analyses and 10 for
162 other analyses) and 10 sea cucumber individuals (both kind of analyses being possible on one
163 individual thanks to the large size of sea cucumber). The final number of individuals was
164 dependent of the available wild organisms on each site. Mussels (n=17; length = 76.75 ± 9.03
165 mm) were found only in St-Florent port. Limpets (n = 13-14 per location; length = 35.24 ± 5.36
166 mm) were found everywhere except in St-Florent port. Sea cucumbers (n = 5-7 per location;
167 length = 154.1 ± 43.77 mm) were collected in the four locations.

168 During the sampling, additional organisms were collected in one location to test the “pool”
169 sampling techniques: 13 mussels (length = 68.56 ± 8.84 mm) and four sea cucumbers (length
170 = 193.25 ± 34.67 mm) were collected in St-Florent and 14 limpets (length = 32.34 ± 4.93 mm)
171 in Île Rousse. Tissues were dissected, pooled and processed following the same method than
172 for the “individual” sampling technique (detailed below for all the analyses). After sampling,
173 individuals were immediately placed in dry ice and shipped to La Rochelle (France) within 48
174 h in order to be analysed. In the lab, half (n = ~7) of the mussels, limpets and sea cucumbers
175 tissues were kept at -20°C for bioaccumulation analyses. The other half was kept at -80°C for
176 all the other analyses.

177

178 A. Biomarkers of exposure (bioaccumulation)

179 a) Trace element assessment

180 The soft tissues of mussel and limpet were hand grinded with a ceramic mortar and pestle. The
181 body wall of sea cucumber was chopped into small pieces. The samples were freeze dried for
182 48-72 h. Then, the samples and the two reference materials (DOLT-5, dogfish liver, and
183 TORT-3, lobster hepatopancreas, National Research Council Canada) were weighted around

184 0.25 mg and digested by addition of 6:2 (v/v) 67-70% HNO₃/34-37% HCl mixture (Fisher,
185 trace metal quality). The digestion process was carried out overnight at room temperature
186 followed by a microwave digestion (30 min with constantly increasing temperature up to 120
187 °C, then 15 min at this temperature). At the end of these steps, the volume of the samples was
188 completed with ultrapure water until 50 mL. Silver (Ag), barium (Ba), cadmium (Cd), cobalt
189 (Co), chromium (Cr), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony
190 (Sb), selenium (Se), tin (Sn) and vanadium (V) contents were analysed using an Inductively
191 Coupled Plasma Optical Emission Spectrometry (ICP-OES) Vista-Pro (Varian Inc., Palo Alto,
192 California, USA). Aluminium (Al), arsenic (As), copper (Cu), iron (Fe) and zinc (Zn) were
193 analysed using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) XSeries 2
194 (ThermoFisher Scientific, Waltham, Massachusetts, USA).

195 Mean recovery rates were 76% (Al / DOLT-5), 55% (Ag / DOLT-5), 93% (As / DOLT-5) and
196 100% (As/ TORT-3), 90% (Cd / DOLT-5) and 95% (Cd / TORT-3), 82% (Co / DOLT-5) and
197 98% (Co / TORT-3), 97% (Cr / TORT-3), 97% (Cu / DOLT-5) and 95 (Cu / TORT-3), 105%
198 (Fe / DOLT-5) and 98% (Fe / TORT-3), 92% (Mn / TORT-3), 87% (Mo / DOLT5) and 85%
199 (Mo / TORT-3), 83% (Ni), 103% (Pb / DOLT-5) and 79% (Pb / TORT-3), 93% (Se / DOLT-
200 5) and 91% (Se / TORT-3), 92% (V / TORT-3) and 95% (Zn / DOLT-5) and 101% (Zn / TORT-
201 3).

202 Limits of quantification were 19.28 (Al), 0.01 (Ag), 0.1 (As), 0.1 (Ba), 0.01 (Cd), 0.01 (Co),
203 0.1 (Cr), 0.96 (Cu), 3.86 (Fe), 0.01 (Mn), 0.1 (Mo), 0.04 (Ni), 0.01 (Pb), 0.04 (Sb), 0.39 (Se),
204 0.02 (Sn), 0.96 (V) and 3.86 (Zn) µg.g⁻¹ of dry weight.

205 The trace element contamination of the water samples was also analysed using ICP-OES
206 (dilution to 1/5 for water samples and seawater standards, in 2 % nitric acid) and ICP-MS
207 (dilution to 1/20 for water samples and seawater standards, in 2 % nitric acid).

208

209 b) Organic pollutants assessment

210 Tissues of organisms (soft tissues of mussel and limpet and body wall of sea cucumber,
211 measured as a pool of seven individuals) were analysed for organic pollutants contents. 22
212 polycyclic aromatic hydrocarbons (PAHs), including the 16 Environmental Protection Agency
213 (EPA) PAHs, 14 polychlorinated biphenyls (PCBs) and seven pesticides were
214 analysed in tissues by stir bar sorptive extraction-thermal desorption-gas chromatography-
215 tandem mass spectrometry (SBSEGC–MS/MS) following Lacroix et al. (2014) method. Tissues
216 were briefly digested by saponification and analytes were extracted for 16 h at 700 rpm using
217 polydimethylsiloxane stir-bars (Twister 20 mm × 0.5 mm, Gerstel). Bars were subsequently
218 analysed using a gas chromatography system Agilent 7890A coupled to an Agilent 7000 triple
219 quadripole mass spectrometer (Agilent Technologies) and equipped with a Thermal Desorption
220 Unit combined with a Cooled Injection System (Gerstel). The GC column was a Restek Rxi-5
221 ms (30 m, 0.25 mm, 0.25 µm). Analytes were quantified relatively to deuterated compounds
222 using a calibration curve ranging from 0.01 ng to 30 ng per bar. Results are expressed as ng.g⁻¹
223 dry weight. Limits of quantification (LOQ) were calculated by the calibration curve method
224 (Shrivastava and Gupta 2011) and were 25 (naphthalene, phenanthrene and PCB 138), five
225 (dibenzothiophene, fluoranthene, 2-methylfluoranthene, chrysene, benzo(b)fluoranthene,
226 benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene,
227 dibenzo(a,h)anthracene, benzo(g,h,i)perylene, PCB 153 and 4-4-DDE), 2.5 (benzothiophene,
228 biphenyl, acenaphthylene, acenaphthene, fluorene, anthracene, pyrene, benzo(a)anthracene,
229 PCB 7, PCB 28, PCB 52, PCB 35, PCB 101, PCB 77, PCB 135, PCB 118, PCB 156, PCB 180,
230 PCB 169, aldrine, isodrine, dieldrine and endrine) and 0.5 (PCB 105, hexachlorobenzene and
231 2-4-DDE) ng.g⁻¹ dry weight. Analytical quality control was done using the Standard Reference
232 Materials 1974c “Organics in Mussel Tissue (*Mytilus edulis*)” provided by the National Institute
233 of Standards (SRM) and Technology (NIST, Gaithersburg, USA).

234

235 B. Biomarkers of effect analyses

236 a) Enzymatic biomarkers

237 Individuals were dissected to keep the digestive gland for mussel, the whole soft tissues for
238 limpets and the body wall for sea cucumber. The tissues were weighted and grounded in liquid
239 nitrogen using a MM400 Retsch[®] (GmbH, Éragny, Luxemburg) mixer mill. Then they were
240 hand homogenized on ice in one volume of phosphate buffered saline (PBS) solution (100 mM,
241 pH 7.5) containing 0.1 % Triton X-100 and one mM ethylenediaminetetraacetic acid (EDTA)
242 to avoid protein degradation. The samples were centrifuged 15 min at 12500 g at 4°C (Sorvall
243 Legeng Micro 17R, ThermoFisher Scientific, Waltham, Massachusetts, USA). The supernatant
244 was collected, divided into aliquots and used for the subsequent biomarkers of effect and protein
245 analyses.

246 The capacity to respond to oxidative stress was evaluated using SOD. SOD activity protocol is
247 based on the inhibition of a superoxide-driven NADH oxidation and was measured following
248 the rate of NADH oxidation at 340 nm (Paoletti et al. 1986). The GST is an enzyme involved
249 in detoxification process. It was determined by measuring the production of GS-DNB at 340
250 nm (Habig et al. 1974). The activities of the indices of glycolysis and gluconeogenesis pathways
251 (PK, PEPCK, and LDH) were determined by measuring the NADH consumption at 340 nm.
252 The method used for PK and LDH followed the Childress and Somero (1979) protocol modified
253 by Bailey et al. (2005). For PEPCK, the Petrescu et al. (1979) method, adapted by Jamieson et
254 al. (1999) was used. However, LDH and PEPCK activities were below the detection levels in
255 mussel and sea cucumber respectively. AChE is widely used to estimate neurotoxic impacts of
256 pollutants, especially organophosphates, carbamates, several heavy metals and surfactants. Its
257 activity was assayed by recording the production of 5-thio-2-nitrobenzoate at 405 nm (Ellman
258 et al. 1961).

259 Finally, the LAC enzyme is involved in immune, antioxidant and detoxification processes
260 (Luna-Acosta et al. 2017a). Its activity was measured according to Luna-Acosta et al. (2010),
261 following the oxidation of p-phenylenediamine at 420 nm.

262 The total protein content was measured with the bicinchoninic acid protein assay kit (Sigma-
263 Aldrich®) that is similar to the Lowry et al. (1951) procedure.

264 All activities measurements were done on a SpectroStar Nano spectrophotometer (BMG
265 labtech, Champigny-sur-Marne, France).

266 All chemicals were obtained from Sigma-Aldrich®. All analyses were performed in technical
267 duplicates for each sample, using standard methods adapted for a microplate reader. For all
268 enzymatic analysis, homogenates were diluted to obtain linear reaction slopes for a minimum
269 of five minutes at 25 °C. Total and specific enzymatic activities were measured and expressed
270 as U ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$) of wet tissue and U. mg^{-1} of protein, respectively.

271

272 b) Glycogen and total lipids

273 Tissues were grounded in liquid nitrogen using a MM400 Retsch® (GmbH, Éragny,
274 Luxemburg) mixer mill and then hand homogenized on ice in one mL of citrate buffer (0.1 M,
275 pH 5). Half of the sample was placed immediately at -80 °C until the total lipid analyses. The
276 other half was incubated for four min in boiling water and then placed at -80 °C.

277 The determination of the total lipid concentration is based on the improved sulfo-phospho-
278 vanillin reaction from Frings et al. (1972). For the glycogen, the sample was incubated with
279 amyloglucosidase resulting in the complete hydrolysis of glycogen to glucose as described in
280 Carr and Neff (1984).

281

282 C. Statistics analyses

283 a) Test on the “pool” technique

284 To test the “pool” technique, the mean of the “individual” samples was compared to with the
285 one of the “pooled” samples. The percentage of difference between these two values was
286 compared for biomarkers of effect and exposure.

287

288 b) Biomarkers

289 Normality and homogeneity of variances were verified by Shapiro and Levene tests. Data were
290 log10 transformed to avoid heteroscedasticity when possible.

291 Pearson's and Spearman's (in case of heteroscedasticity) correlations between total and specific
292 enzymatic activity responses were verified for all enzymes.

293 In limpet and sea cucumber, one-way ANOVAs (or non-parametric Kruskal-Wallis tests in case
294 of heteroscedasticity) were used to test the effects of location on the biomarkers of effect and
295 trace element bioaccumulation, followed by respectively Tukey or Mann-Whitney Wilcoxon
296 post-hoc test when a significant effect was found ($P < 0.05$). Due to many values below the
297 limit of quantification (<LOQ), testing the difference between location was not possible for the
298 organic pollutants' bioaccumulation data. To determine the most important parameters in our
299 multi-parameter analysis, principal component analysis (PCA) was performed on trace element
300 and organic contaminants concentration in tissue of limpet and sea cucumber grouped per
301 location.

302 Finally, for limpet and sea cucumber, the "Integrated Biomarker Response" (IBR, (Devin et al.
303 2014)) was calculated using the response of biomarkers of effect (results from the “individual”
304 sampling technique). On R software, the functions from the package IBRtools were used (Devin
305 et al. (2014) based on Beliaeff and Burgeot (2002)).

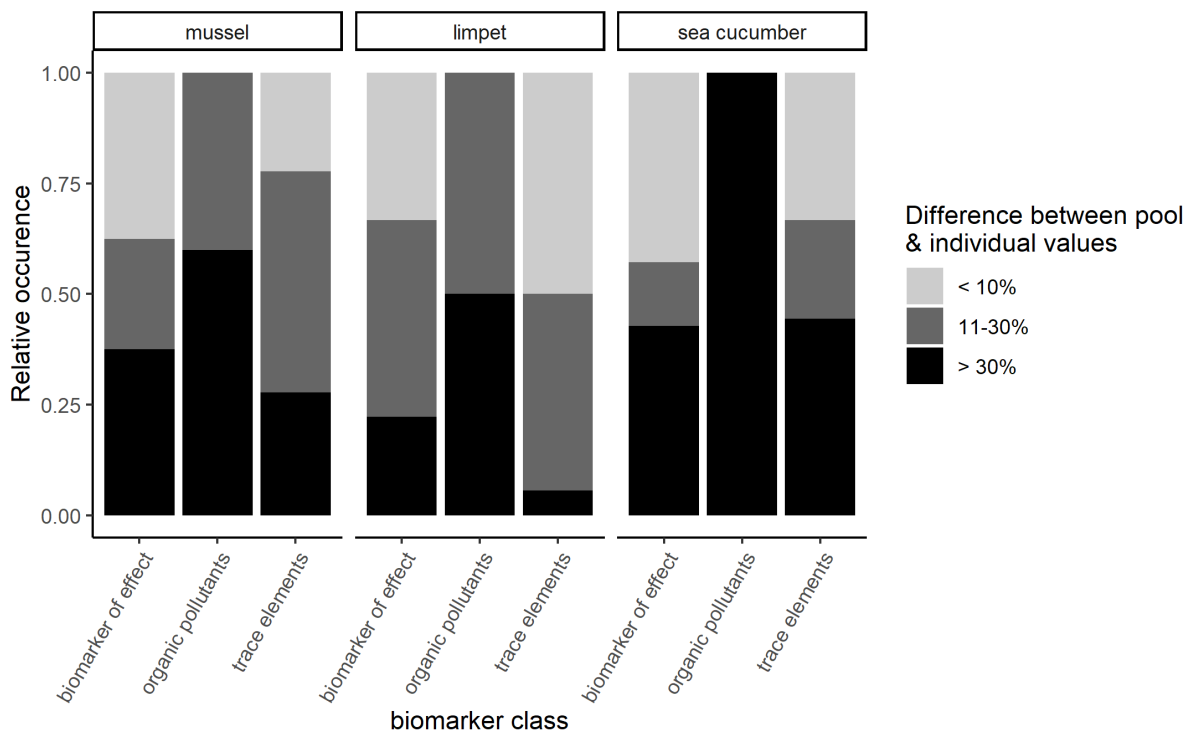
306

307 **Results**

308 A. Test on the “pool” technique

309 For all the biomarkers, the difference between the value obtained for “pooled” samples and the
310 average of the values obtained for each “individual” samples were classified into three
311 categories: < 10% of difference, 10-30% of difference and > 30% of difference (Figure 2).

312



313

314 **Fig. 2** Occurrence of the different class of difference (in %) between « pool » and
315 « individual » techniques, for biomarker of effect and exposure (trace element and organic
316 pollutant bioaccumulation) in the three marine molluscs

317

318 The difference between the “pool” and the “individual” samples varied according to the species
319 and class of biomarkers. In mussels, the difference between “pool” and “individual” techniques
320 was less than 30% in 6.25, 7.22 and four times out of 10 for the biomarkers of effect, the metallic
321 trace elements and the organic pollutants bioaccumulation respectively. For limpet, the same

322 was observed 7.8, 9.4 and five times out of 10 and for sea cucumber, it was 5.7, 5.5 and 0 times
 323 out of ten. On the contrary to the other biomarker classes, the difference was superior or equal
 324 to 30% in all species for the organic pollutants.

325

326 B. Trace element contamination of water

327 Among the 18 metallic trace elements measured in water samples (Table 1), only five (Co, Cu,
 328 Mn, Mo and Pb) were above the limit of quantification. The highest values for Cu and Mn were
 329 observed in St-Florent and the one of Mo was detected in Calvi. The higher number of metallic
 330 trace elements was detected in Île Rousse. No trace element contamination was observed at
 331 STARESO.

332

333 **Table 1:** Trace element concentration ($\mu\text{g/L}$) in water sample collected in the different
 334 locations in January. Grey cells indicate values higher than the limit of detection.

	STARESO	Calvi	Île Rousse	St-Florent
Ag	< 10	< 10	< 10	< 10
Al	< 200	< 200	< 200	< 200
As	< 10	< 10	< 10	< 10
Ba	< 10	< 10	< 10	< 10
Cd	< 2	< 2	< 2	< 2
Co	< 1	1,0	< 1	< 1
Cr	< 10	< 10	< 10	< 10
Cu	< 2	2,6	5,5	12,3
Fe	< 2500	< 2500	< 2500	< 2500
Mn	< 1	< 1	< 1	18,94
Mo	< 10	12,7	11,0	< 10
Ni	< 100	< 100	< 100	< 100
Pb	< 1	< 1	1,9	< 1
Sb	< 1	< 1	< 1	< 1
Se	< 40	< 40	< 40	< 40
Sn	< 2	< 2	< 2	< 2
V	< 100	< 100	< 100	< 100
Zn	< 2500	< 2500	< 2500	< 2500

335

336 C. Biomarkers of exposure

337 For the trace element contamination (Table 2), differences between location were statistically
338 tested only for limpet and sea cucumber as wild mussel was found only in St-Florent. While the
339 difference between location were found only for Mn ($P < 0.001$) regarding sea cucumber,
340 differences were observed for Al, As, Ba, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn in limpet.
341 The contamination trend in limpet differed according to the trace element: for Al, Cu, Fe, Mn,
342 Se ($P < 0.001$) and Co ($P < 0.01$), higher levels were found in Calvi and Île Rousse compared
343 to STARESO; for Ba ($P < 0.05$) and Pb ($P < 0.001$), the contamination level is lower in
344 STARESO compared to those in Île Rousse; for Zn and V ($P < 0.001$), Calvi was the most
345 contaminated location; for Cr and Ni ($P < 0.001$), STARESO and Calvi contamination was
346 higher than Île Rousse; for As ($P < 0.001$), Calvi presented a lower contamination than Île
347 Rousse, and STARESO presented intermediate levels. For all the other metallic trace elements
348 (Ag, As, Cd, Mo, Sb, Sn), the contamination registered in organisms was similar among
349 locations.
350

351 **Table 2:** Trace element concentration ($\mu\text{g/g}$ of wet tissue) in soft tissue of mussel and limpet and body wall of sea cucumber collected in North
 352 Corsica in January 2020 (mean \pm standard deviation, $N=7$). Letters indicate significant difference between the locations ($P < 0.05$) within the
 353 same species.

	mussel		limpet				sea cucumber				
	St Florent (individual)	St Florent (pool)	STARESO	Calvi	Île Rousse (individual)	Île Rousse (pool)	STARESO	Calvi	Île Rousse	St Florent (individual)	St Florent (pool)
Al	363.17 \pm 119.42	441.35	61.3 \pm 8.36 ^b	382.54 \pm 150.34 ^a	497.18 \pm 186.09 ^a	491.37	59.33 \pm 8.71	101.72 \pm 54.36	70.67 \pm 22	115.33 \pm 72.28	99.35
As	15.89 \pm 3.38	10.96	30.4 \pm 11.56 ^{ab}	16.21 \pm 2.02 ^b	41.28 \pm 11.53 ^a	33.98	19.78 \pm 11.61	8.85 \pm 3.5	14.43 \pm 7.28	26.89 \pm 28.06	18.48
Ba	1.65 \pm 0.52	1.88	0.53 \pm 0.2 ^b	1.78 \pm 1.01 ^{ab}	4.48 \pm 4.34 ^a	3.96	1.47 \pm 0.4	1.44 \pm 0.57	1.56 \pm 0.65	1.2 \pm 0.66	1.35
Cd	0.47 \pm 0.1	0.43	1.95 \pm 1.07	0.46 \pm 0.09	0.8 \pm 0.24	0.78	0.03 \pm 0	0.07 \pm 0.07	0.04 \pm 0.02	0.08 \pm 0.07	0.05
Co	1.15 \pm 0.21	1.24	0.21 \pm 0.09 ^b	0.39 \pm 0.08 ^a	0.33 \pm 0.1 ^a	0.32	0.21 \pm 0.07	0.2 \pm 0.07	0.22 \pm 0.09	0.48 \pm 0.57	0.28
Cr	5.09 \pm 1.59	5.38	7.04 \pm 4 ^a	10.09 \pm 1.92 ^a	2.42 \pm 0.94 ^b	2.89	0.58 \pm 0.33	0.71 \pm 0.49	0.33 \pm 0.14	1.52 \pm 1.09	1.40
Cu	28.29 \pm 7.02	40.10	7.23 \pm 2.43 ^b	110.18 \pm 21.6 ^a	55.93 \pm 28.39 ^a	55.92	3.12 \pm 1.8	5.99 \pm 3.5	5.07 \pm 3.29	5.03 \pm 3.9	4.01
Fe	760.59 \pm 218.87	925.45	579.69 \pm 256.92 ^b	2091.53 \pm 405.59 ^a	2041.55 \pm 708.44 ^a	2503.80	41.11 \pm 18.27	130.26 \pm 108.53	121.35 \pm 80.46	213.09 \pm 210.65	156.01
Mn	28.71 \pm 7.91	39.84	2.72 \pm 0.9 ^b	6.21 \pm 1.8 ^a	6.08 \pm 1.65 ^a	5.26	0.78 \pm 0.3 ^a	1.64 \pm 0.77 ^{ab}	1.14 \pm 0.7 ^a	29.11 \pm 55.3 ^b	7.13
Mo	1.77 \pm 0.47	1.47	0.76 \pm 0.27	0.62 \pm 0.09	0.72 \pm 0.14	0.75	2.98 \pm 1.85	15.89 \pm 22.33	3.32 \pm 2.85	7.11 \pm 4.97	7.05
Ni	4.96 \pm 1.45	5.44	4.92 \pm 1.37 ^a	5.97 \pm 1.27 ^a	1.95 \pm 0.49 ^b	1.95	2.62 \pm 0.96	2.17 \pm 0.86	2.56 \pm 1.11	2.72 \pm 1.54	2.83
Pb	1.15 \pm 0.29	1.36	1.3 \pm 0.42 ^b	4.36 \pm 1.03 ^{ab}	7.04 \pm 2.71 ^a	6.73	1.68 \pm 0.85	1.84 \pm 0.64	1.34 \pm 0.32	1.33 \pm 1.16	0.60
Se	4.83 \pm 0.97	4.88	0.86 \pm 0.31 ^b	1.46 \pm 0.08 ^a	1.43 \pm 0.15 ^a	1.46	5.56 \pm 2.54	4.48 \pm 2.46	3.18 \pm 1.5	6.06 \pm 0.83	5.68
V	1.46 \pm 0.43	1.73	0.93 \pm 0.13 ^b	5.43 \pm 2.65 ^a	2.6 \pm 0.77 ^b	3.16	0.7 \pm 0.04	0.7 \pm 0.03	0.72 \pm 0.02	1.05 \pm 0.9	0.67
Zn	156.41 \pm 59	189.99	60.65 \pm 21.68 ^c	218.88 \pm 22.82 ^a	132.84 \pm 21.11 ^b	143.93	37.09 \pm 11.45	46.87 \pm 15.57	39.68 \pm 12.86	43.04 \pm 6.24	43.98
Ag	0.02 \pm 0.01	0.03	0.36 \pm 0.19	0.07 \pm 0.03	0.05 \pm 0.03	0.04	0.02 \pm 0.02	0.04 \pm 0.08	0.03 \pm 0.07	0.01 \pm 0	0.01
Sn	0.03 \pm 0.02	0.01	0.02 \pm 0	0.02 \pm 0	0.03 \pm 0.02	0.02	0.38 \pm 0.66	0.27 \pm 0.17	0.34 \pm 0.2	0.17 \pm 0.22	0.03
Sb	0.04 \pm 0.01	0.03	0.04 \pm 0.01	0.09 \pm 0.04	0.06 \pm 0.02	0.09	0.03 \pm 0	0.05 \pm 0.05	0.03 \pm 0	0.03 \pm 0.01	0.03

354

355 Organic pollutants bioaccumulation (Table 3) was only detected in St-Florent for mussel and
356 sea cucumber and in Île Rousse for limpet. Mussels accumulated more organic pollutants (14
357 HAPs, eight PCBs and one pesticide) than limpets (8 HAPs) or sea cucumbers (5 HAPs).
358 Concerning the bioaccumulation of the different organic pollutant categories, mussel was the
359 only organism to accumulate PCBs and pesticides.
360

361 **Table 3:** Organic pollutant concentration ($\mu\text{g/g}$ of wet tissue) in soft tissue of mussel and limpet and body wall of sea cucumber collected in North
 362 Corsica in January (mean \pm standard deviation, N=1-7). <LD = below the detection limit.

	mussel		limpet				sea cucumber				
	St Florent (individual)	St Florent (pool)	STARESO	Calvi	Île Rousse (individual)	Île Rousse (pool)	STARESO	Calvi	Île Rousse	St Florent (individual)	St Florent (pool)
naphthalene	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
benzothiophene	<LD	<LD	<LD	<LD	4.92 \pm 0.72	<LD	<LD	<LD	<LD	<LD	<LD
biphenyl	<LD	<LD	<LD	<LD	4.61 \pm 2.03	3.02	<LD	<LD	<LD	5.52	<LD
acenaphthylene	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
acenaphthene	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
fluorene	<LD	<LD	<LD	<LD	3.89	2.98	<LD	<LD	<LD	4.15	<LD
dibenzothiophene	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
phenanthrene	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
anthracene	3.63	<LD	<LD	<LD	3.18	<LD	<LD	<LD	<LD	<LD	<LD
fluoranthene	6.58	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
2-methylfluoranthene	9.80 \pm 0.85	8.47	<LD	<LD	11.51 \pm 3.07	14.47	<LD	<LD	<LD	<LD	<LD
pyrene	4.06	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
benzo(a)anthracene	10.36 \pm 9.38	3.64	<LD	<LD	<LD	2.69	<LD	<LD	<LD	<LD	<LD
chrysene	18.35 \pm 15.55	8.12	<LD	<LD	7.72 \pm 1.59	12.39	<LD	<LD	<LD	<LD	<LD
benzo(b)fluoranthene	16.98	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
benzo(k)fluoranthene	18.71	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
benzo(e)pyrene	10.23 \pm 6.73	<LD	<LD	<LD	5.62 \pm 0.64	10.88	<LD	<LD	<LD	<LD	<LD
benzo(a)pyrene	9.41	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
perylene	9.24 \pm 4.35	5.36	<LD	<LD	<LD	<LD	<LD	<LD	<LD	8.28 \pm 2.19	5.30
indeno(1,2,3-cd)pyrene	5.75	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
dibenzo(a,h)anthracene	12.65	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	8.52	<LD
benzo(g,h,i)perylene	12.67 \pm 1.84	9.88	<LD	<LD	7.89 \pm 3.98	7.05	<LD	<LD	<LD	9.17 \pm 3.29	<LD

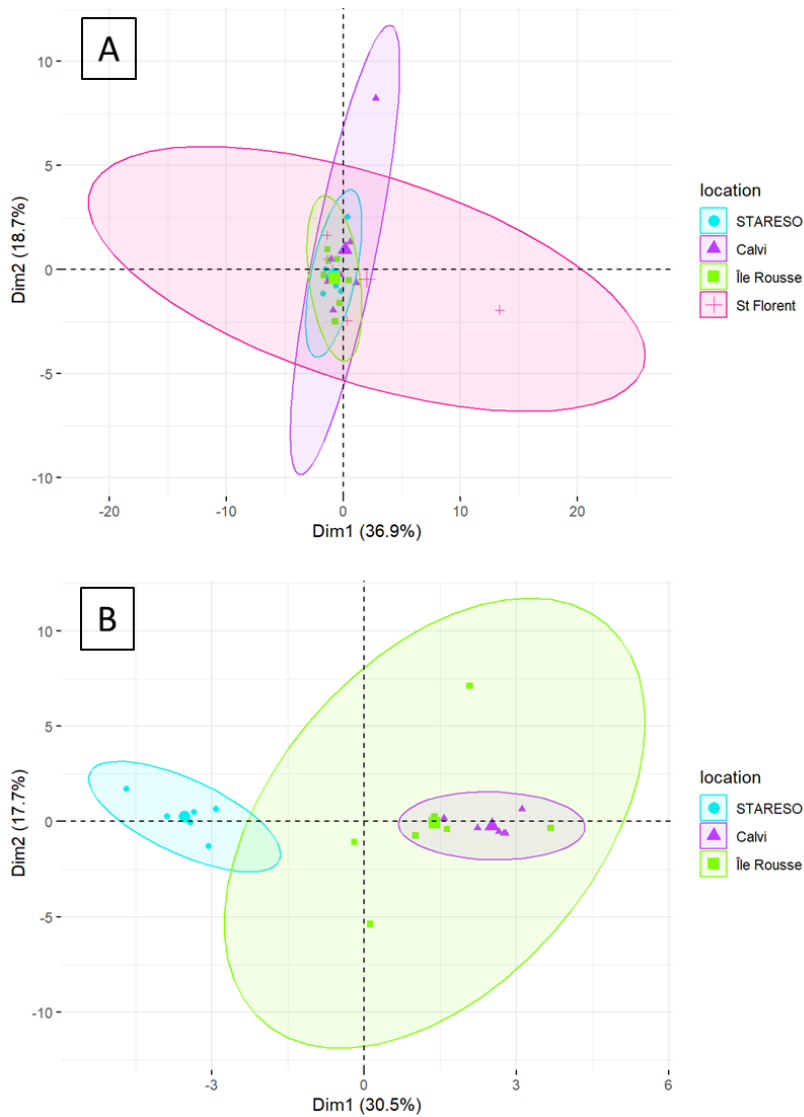
PCB 7	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 28	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 52	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 35	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 101	2.80	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 77	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 135	3.27	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 118	4.87	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 153	7.29 ± 2.03	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 105	3.19	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 138	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 156	5.76	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 180	8.63	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 169	4.20	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
hexachlorobenzene	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
aldrine	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
isodrine	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
2-4-DDE	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
4-4-DDE	5.31	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
dieldrine	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
endrine	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD

364 No organic pollutants were detected in limpet collected in STARESO and Calvi or in sea
365 cucumber collected in STARESO, Calvi and Île Rousse. However, organic pollutants
366 concentrations in tissues of organisms were below the Environmental Quality Standards (EQS)
367 defined by the 2013/39/EU directive for biota.

368

369 When analysing the results of all contaminants (trace element and organic contaminants) in sea
370 cucumber tissues, the PCA principal component (Dim1) explained 36.9% while the second
371 principal component (Dim2) explained 18.7 % of the total variation. It was difficult to
372 determine the most important factors for the model because of the high number of parameters
373 (Figure 3A). Contaminant concentrations in tissue from St-Florent seemed to be more explained
374 by Dim1. Dim1 was related to concentrations of Cd, Co and Mn. In the other locations,
375 contaminant concentrations in tissues seemed to be better explained by Dim2. Dim2 is mainly
376 related to Mo, Sb, Ag, Ba and Ni (Figure 3A, supplementary material 1). In limpet, the PCA
377 principal component (Dim1) explained 30.7% while the second principal component (Dim2)
378 explained 17.7 % of the total variation. While contaminants concentration in tissue from Île
379 Rousse seemed to be explained by both the Dim1 and Dim2, contaminants concentration in
380 tissue from STARESO and Calvi seemed to be more explained by Dim1.

381



382

383 **Fig.3** PCA results for trace element and organic contaminants in (A) sea cucumber and (B)
 384 limpet tissues per location

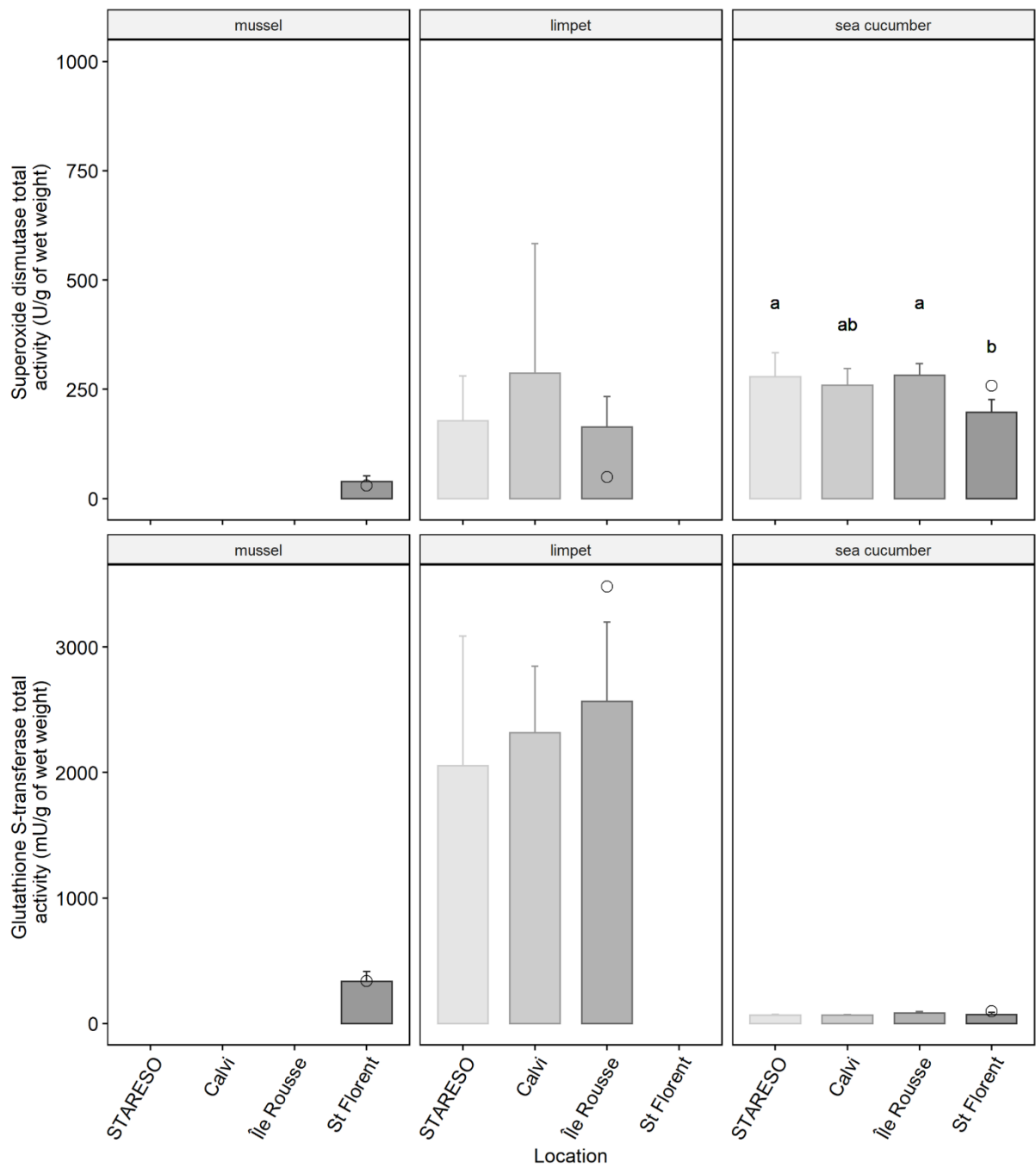
385

386 D. Biomarkers of effect

387 Specific and total activities measured in mussel, limpet and sea cucumber were significantly
 388 correlated (R between 0.89 and one depending of the enzyme, $P < 0,001$). Total activities and
 389 specific activities can be found in Supplementary Tables 1.

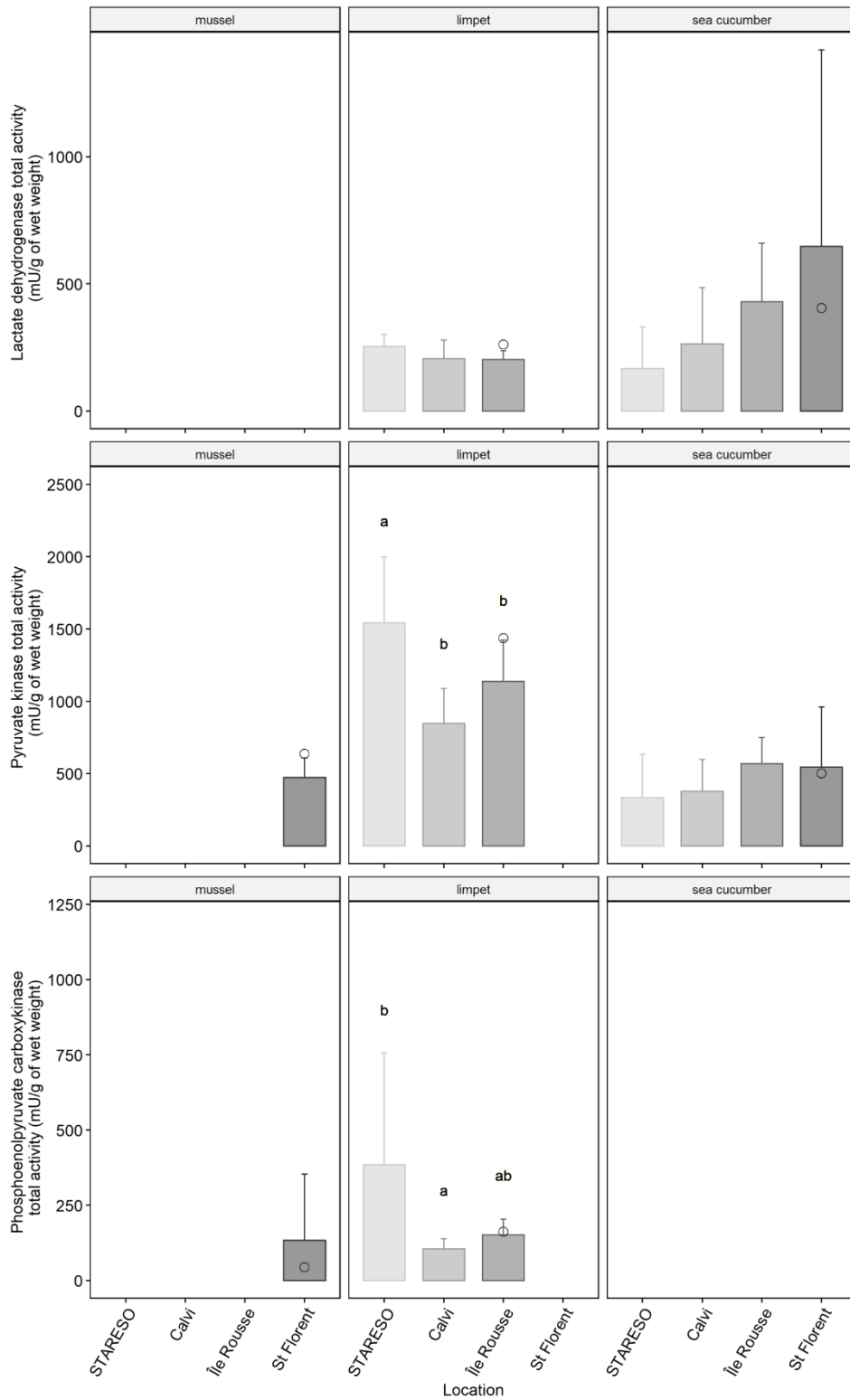
390

391 The response of the antioxidant system varied according to the species (Figure 4). In limpet the
 392 SOD and GST activities were much higher than those detected in mussel (and in sea cucumber
 393 for GST). The effect of location was significant for sea cucumber SOD activity. Its SOD activity
 394 was 1.2 times higher ($P < 0.01$) in individuals collected in STARESO and Île Rousse than in
 395 those from St-Florent.
 396



397

398 **Fig. 4** Total enzymatic activity of superoxide dismutase (U/g of wet tissue) and glutathione S-
399 transferase (mU/g of wet tissue) in digestive gland of mussel, soft tissue of limpet and body
400 wall of sea cucumber collected in North Corsica in January (mean \pm standard deviation).
401 Different letters indicate significant difference between the location, within the same species
402 ($P < 0.05$). Circles indicate the value for the pooled data collected in the same location.
403
404 LDH, PK and PEPCK (Figure 5) are respectively involved in fermentation, glycolysis and
405 neoglycolysis. Significant variations of their activities were only observed for limpets. In
406 mussel and sea cucumber, the activity of these three enzymes was similar. In limpet collected
407 in STARESO, the PK activity was significantly higher than in Calvi and Île Rousse, and the
408 PEPCK activity was significantly higher than in Calvi.
409



410

411

412

413

414

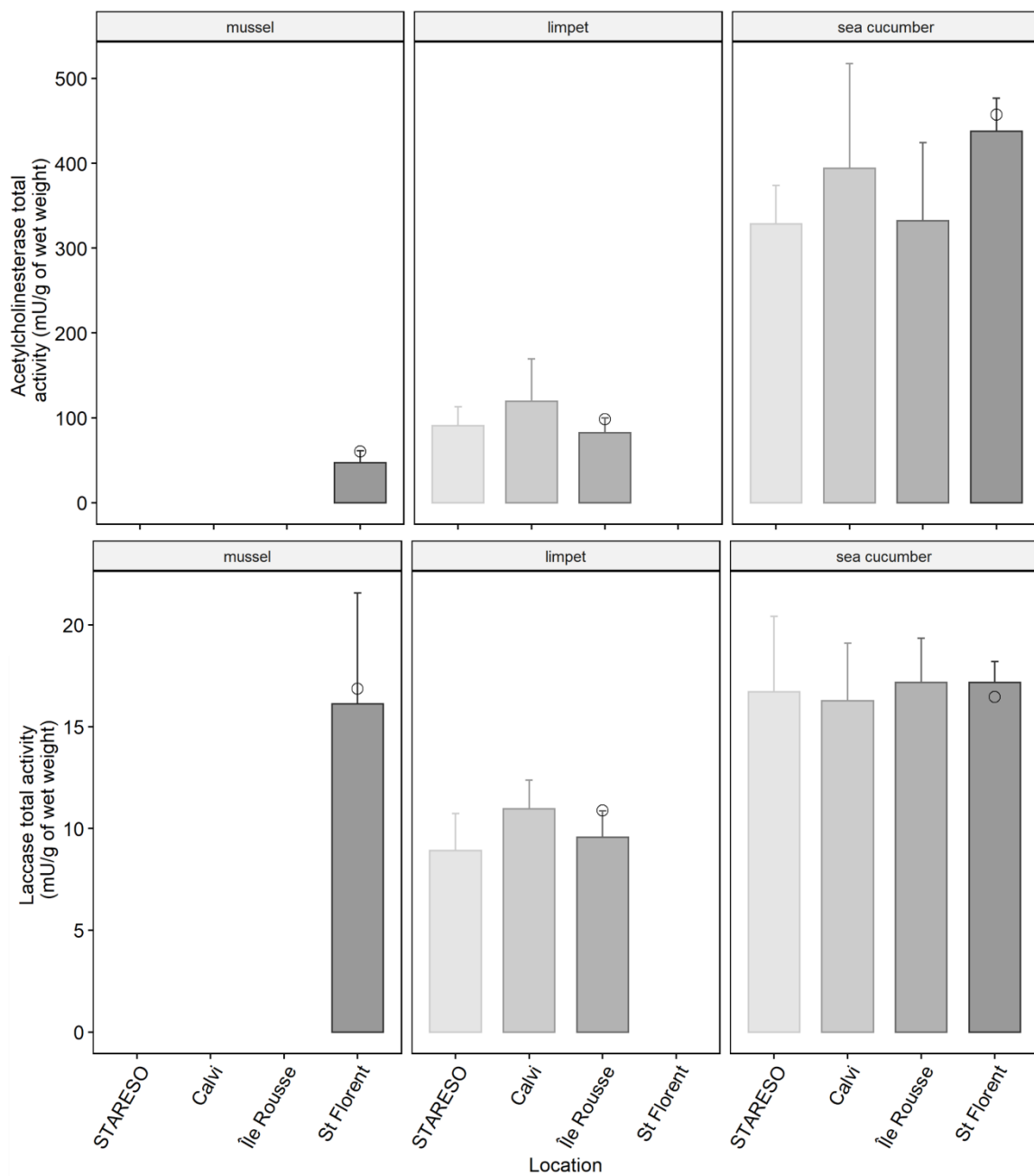
Fig. 5 Total enzymatic activity of lactate dehydrogenase, pyruvate kinase and phosphoenolpyruvate carboxykinase (mU/g of wet tissue) in digestive gland of mussel, soft tissue of limpet and body wall of sea cucumber collected in North Corsica in January (mean \pm standard deviation). Different letters indicate significant difference between the location,

415 within the same species ($P < 0.05$). Circles indicate the value for the pooled data collected in
416 the same location

417

418 The activity of AChE (Figure 6), used as an indicator of neurotoxicity, was similar according
419 to the location for all three organisms. The same was noted for the activity of LAC, enzyme
420 involved in immunity processes.

421



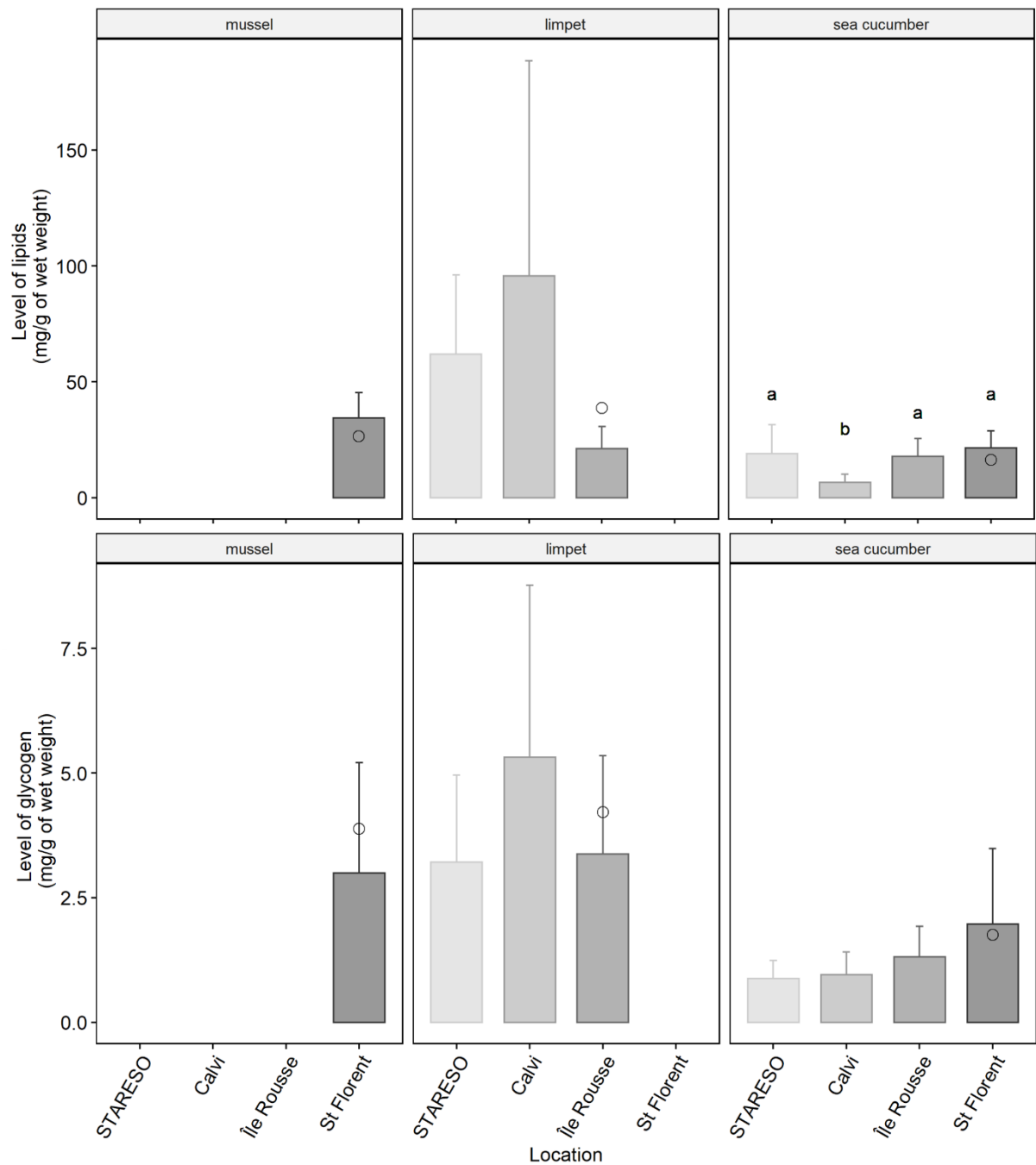
422

423 **Fig. 6** Total enzymatic activity of acetylcholinesterase and laccase (mU/g of wet tissue) in
424 digestive gland of mussel, soft tissue of limpet and body wall of sea cucumber collected in
425 North Corsica in January (mean \pm standard deviation). Different letters indicate significant
426 difference between the location, within the same species ($P < 0.05$). Circles indicate the value
427 for the pooled data collected in the same location

428

429 The lipid and glycogen levels (Figure 7) presented approximately the same levels in mussel and
430 in limpet. For limpet, no differences between locations were noted either for the lipids or
431 glycogen contents. In sea cucumber, the glycogen content was similar among location while
432 the lipids content was significantly lower in Calvi compared to the other locations.

433



434

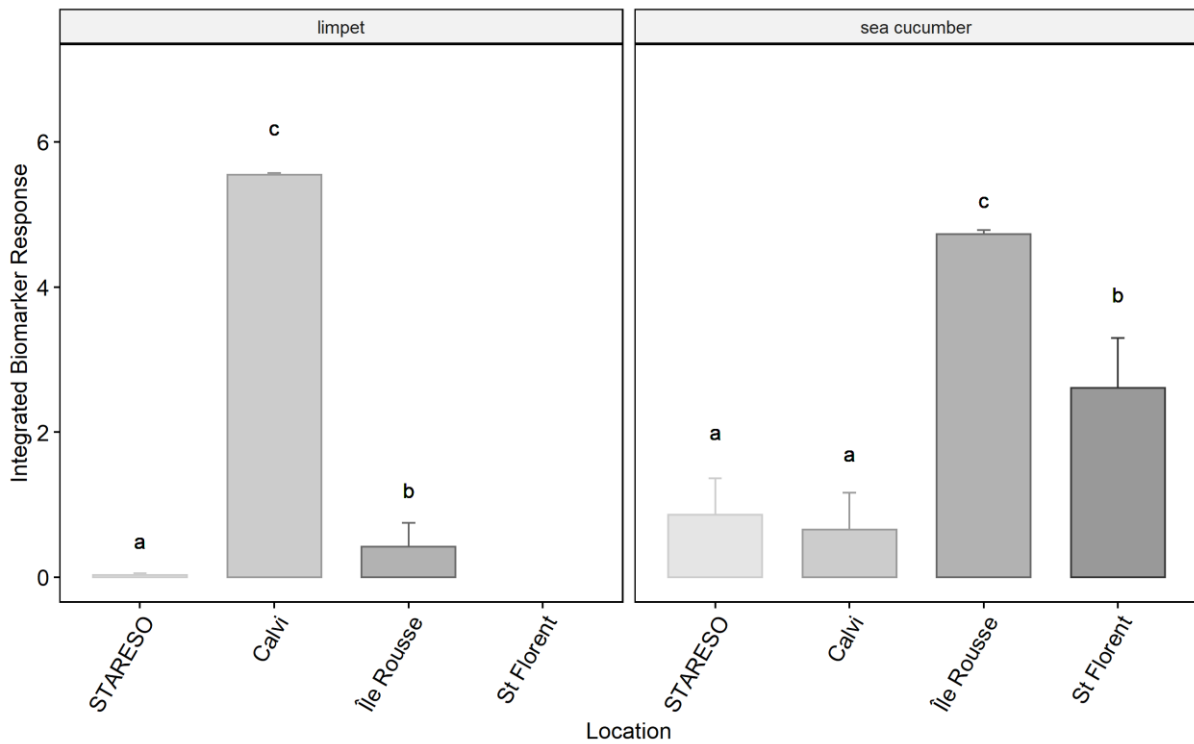
435 **Fig. 7** Total lipids and glycogen concentrations (mg/g of wet tissues) in digestive gland of
 436 mussel, soft tissue of limpet and body wall of sea cucumber collected in North Corsica in
 437 January (mean \pm standard deviation). Different letters indicate significant difference between
 438 the location, within the same species ($P < 0.05$). Circles indicate the value for the pooled data
 439 collected in the same location

440

441 E. Integrated biomarker response (IBR)

442 The IBR calculated using the biomarkers of effect response gave variable results according to
443 the organism (Figure 8). In limpet, the IBR value was the highest in Calvi, the lowest in
444 STARESO and intermediate in Île Rousse. In sea cucumber, the lowest IBR values were found
445 in STARESO and Calvi, the greatest values were found in Île Rousse.

446



447

448 **Fig. 8** Integrated Biomarker Responses (IBR, mean) calculated for limpet and sea cucumber
449 using the biomarker of effects. Different letters indicate significant difference between the
450 location, within the same species ($P < 0.05$)

451

452 **Discussion**

453 Our study provides the first ecological data based on passive biomonitoring from three port
454 areas in North Corsica. The organisms were collected during the three years of the QUAMPO
455 (QUALity of the marine environment in the Mediterranean PORT areas) project (2019-2022).

456 They were analysed considering a multibiomarkers approach (indicators of stress, energetic
457 metabolism, neurotoxicity, immunity, metallic trace elements/organic pollutants
458 bioaccumulation).

459

460 In accordance with our first hypothesis, the results of this study validate the use of the “pool”
461 sampling technique. This work therefore confirms the conclusion of several other studies
462 focusing on the analyses of organic pollutant in herring gulls (*Larus argentatus*) (Turle and
463 Collins 1992) or on the analyses of metallic trace elements and biomarker of effect on black
464 scallop (*Mimachlamys varia*) collected in port areas (Breitwieser et al. 2018). Both authors
465 showed the reliability of the “pool” sampling technique, as the means of individual analyses
466 and those of pooled samples was lower than the normal analytical variation. A validation was
467 however necessary in the present study because the results might differ according to the studied
468 organisms and the considered biomarkers. Here, the “pool” technique was especially reliable
469 for Al, Cd, Co, Cu, Mo, Ni, Pb, Se and Zn accumulations in limpet; for LAC activities, Cd, Co,
470 Cr and Se accumulations in mussel; and for AChE and LAC activities, Ag, Cr, Mo, Ni, Se and
471 Zn accumulations in sea cucumber. The reliability of the “pool” technique for metallic trace
472 element accumulation was especially observed in limpet. However, the “pool” sampling
473 techniques is not adapted to the organic pollutant bioaccumulation analyses in our study. This
474 is mainly because most of the analysed organic pollutants are close to or below the detection
475 limit.

476 Considering this, the “pool” technique allows researchers to obtain reliable results at lower
477 costs (Breitwieser et al. 2018). This can be advantageous when the analyses are quite expensive
478 (trace element or organic pollutants analyses for example), when the population is composed
479 of a limited number of individuals, to gain time in laboratory analyses or to reduce the number
480 of organisms (3 R’s: Reduce, Reuse, Recycle). This technique has however some disadvantages

481 as it does not allow to precisely assess the impact of a mix of contaminants nor to detect extreme
482 or abnormal values as with inter-subject studies. Also, it may have negative consequences when
483 the biological variation is important or not known (Bignert et al. 1993; Bignert et al. 1994).

484

485 Our second hypothesis supposed that both mussel and limpet would give similar results while
486 sea cucumber would be more contaminated. This was shown to be wrong by our results. In fact,
487 biomarkers gave more responses in limpet than in sea cucumber. They allowed statistical
488 discrimination of the studied locations. Mussel had a similar response as limpet, however,
489 mussel are only found in St-Florent, where the primary production is enhanced by freshwater
490 inputs coming from the Aliso River. In the other locations, the oligotrophic conditions are not
491 suitable for the installation of mussel. In mussel, it is then difficult to affirm that the biomarker
492 response reflects the pollution on the locations rather than the starvation effect. Considering
493 this and despite the bioindicative capacity of mussel shown in many studies (Caricato et al.
494 2010; Lehtonen et al. 2019; Uluturhan et al. 2019), mussel is not adapted to the environmental
495 conditions of North Corsica port areas. Mussel should only be used in complementarity with
496 other organisms. In sea cucumber, the only effect of location observed on the biomarkers of
497 effect was for the lipids content and SOD activity. Compared to the other locations, lipid
498 contents were lower in Calvi. This might reflect a lower food supply at this location. The SOD
499 activity was higher in STARESO and Île Rousse compared to the one in St-Florent. SOD is
500 involved in antioxidative defences, and this response could reflect more stress in STARESO
501 and Île Rousse. However, the significant difference in SOD activity was only detected in total
502 activity and not in specific activity, meaning there is no significant difference at a protein level.
503 The variation in SOD total activity is not confirmed by the trace element bioaccumulation in
504 sea cucumber: most metallic trace elements (except Mn) have similar levels among location.
505 Mn bioaccumulation varied across sites and St-Florent seems to be the most polluted one. The

506 higher metallic contamination in St-Florent compared to the other locations is also confirmed
507 by the multivariate analysis that includes all contaminants. The biomarkers of exposure and
508 biomarkers of effect gave contradictory responses in St-Florent. This could be explained by the
509 presence of some HAPs detected or even of other pollutants not analysed in the present study
510 (e.g., emerging contaminants). Considering these results, the sea cucumber might not be an
511 efficient bioindicator species in North Corsica. However, previous studies demonstrated the
512 reliability of sea cucumber as bioindicator (Aydin et al. 2017; Parra-Luna et al. 2020; Turk
513 Culha et al. 2016), especially for bioaccumulation analyses. For example, Turk Culha et al.
514 (2016) detected average levels of metallic trace elements equal to 0.18 $\mu\text{g/g}$ for Cd, 2.43 $\mu\text{g/g}$
515 for Cu, 2.09 $\mu\text{g/g}$ for Pb, 14.58 $\mu\text{g/g}$ for Ni, 16.86 $\mu\text{g/g}$ for Zn, and 73.46 $\mu\text{g/g}$ for Fe in sea
516 cucumber (*H. tubulosa*) sampled in the Dardanelles Strait (Turkey). In our study, for the similar
517 metallic trace elements, the levels were lower for Cd, Pb and Ni (respectively 0.05; 1.55 and
518 2.52 $\mu\text{g/g}$) and higher for Cu, Zn and Fe (respectively, 4.8; 41.67 and 126.45 $\mu\text{g/g}$) in the same
519 species. Our results confirm the bioaccumulative capacities of sea cucumber, but the lack of
520 correlation with the biomarker of effects should be considered when using this species as
521 bioindicator. The tissue analysed in the present study (body wall of sea cucumber), could also
522 impacted our results. The sensibility of body wall might be lower compared to the one of other
523 tissues such as respiratory tree or intestine (Aydin et al. 2017).

524 The limpet seems to be the most promising organism to monitor the effects of pollutants in
525 North Corsica port areas. In our study, it bioaccumulates more metallic trace elements in Calvi
526 and Île Rousse (significant difference between location for Al, As, Ba, Co, Cu, Cr, Fe, Mn, Pb,
527 Se, V and Zn) and some HAPs (benzothiophene, biphenyl, fluorene, anthracene, 2-
528 methylfluoranthene, chrysene, benzo(e)pyrene and benzo(g,h,i)perylene) in Île Rousse.
529 Limpets also seem more impacted in Calvi and Île Rousse than in STARESO. For example, PK
530 activity is lower in Calvi and Île Rousse, and PEPCK activity is lower in Calvi, compared to

531 those in STARESO. The biomarkers of exposure response are correlated with the biomarkers
532 of effect response (for both total and specific activities). Our results confirm the reliability of
533 limpets as a bioindicator, their sensibility to detect contamination difference among locations
534 and the possibility to use them as a good substitute or complement to mussels for biomonitoring
535 marine pollution. Our results verified the bioaccumulative capacities of limpets for various
536 contaminants (but especially for Cd, Pb, Cu & Zn) demonstrated by several authors (Conti et
537 al. 2017; Mbandzi et al. 2022; Pérez et al. 2019). From an ecological point of view, limpet is as
538 interesting as a bioindicator species as mussel: its biomarkers of effects responses are linked to
539 those of the biomarker of exposure (Gharred et al. 2019; Gouveia et al. 2019; Pérez et al. 2019;
540 Silva et al. 2018).

541

542 Considering the complexity of biochemical responses to chronic pollution, the use of a
543 multibiomarkers approach is strongly recommended (Caricato et al. 2010). The IBR is a method
544 combining several biomarker responses of effect into a general “stress index”. In the present
545 study, it was calculated for limpets and sea cucumbers from the different locations. It allowed
546 for the identification of the most impacted location. Firstly, for both species, the IBR was the
547 lowest in STARESO. This underscores the absence of trace element or organic pollutants
548 contamination and confirms the use of STARESO as a reference location. The highest IBR
549 values were not located in the same port for limpet and sea cucumber. The IBR calculated with
550 the limpet responses shows that Calvi seems the most impacted location while the one
551 calculated with the sea cucumber responses shows higher values for Île Rousse and St-Florent.
552 Previous studies used the IBR to provide indication of the environmental stress caused by
553 environmental (Serafim et al. 2012) or anthropogenic (Cao et al. 2019; Ji et al. 2019; Luna-
554 Acosta et al. 2017b; Silva et al. 2018) factors. However, the biological responses must be
555 interpreted cautiously and in combination with chemical data to avoid misinterpretation (Cravo

556 et al. 2012). The calculation of the IBR is highly dependent on a number of a priori choices
557 (Beliaeff and Burgeot 2002). It will not explain the biological meaning, but will rather provide
558 the mathematical relations between the biological data (Ji et al. 2019). In our study, the IBR
559 gives us a first integrated view of the situation in the North Corsica port. As already shown in
560 previous studies, the IBR is a promising tool for scientists and managers to classify the toxic
561 potential of coastal areas (Beliaeff and Burgeot 2002; Benali et al. 2015).

562

563

564 Finally, in accordance with our last hypothesis, the pollution on the considered areas is still low
565 compared to other bigger port areas in the Mediterranean Sea (Galvani et al. 2006). This is
566 mainly due to the small size of the ports (between 250 and 900 boats) considered in the present
567 study. Despite some anthropic pressures registered close to the ports (such as boats rental
568 agencies), the chemical contamination was still below the reglementary threshold values.

569 For future research, it will be necessary to obtain more data on environmental parameters and
570 on the port contamination. This could be done by measuring environmental conditions
571 (temperature, salinity, oxygen or chlorophyll-a), by collecting more water samples and by
572 adding sediments analyses. Furthermore, it will be necessary to integrate more biomarker data
573 (for example other contaminants such as tributyltin) or condition index to consider the
574 intraspecific variability of wild organisms and how their feeding mode influence their response
575 to chemical exposure. Longer biomonitoring is also needed to improve the evaluation of the
576 contaminated locations. Finally, to more precisely test the bioindicative capacities of species,
577 some experimental studies with controlled conditions would be essential.

578

579 **Conclusion**

580 This study is the first to give information on the ecological quality of the port areas in North
581 Corsica. The biomonitoring carried out using three bioindicator species (*M. galloprovincialis*,
582 *Patella* sp. and *H. tubulosa*) demonstrated that the choice of a species adapted to the local
583 environment is of primary importance for efficient biomonitoring. In North Corsica, the limpet
584 is present in most locations in comparison to the mussel, and it responds better than the sea
585 cucumber. For future biomonitoring, it would be very interesting to use the limpet in
586 combination with the mussel to have a global view of the ecological quality of the water in the
587 area. The combination between limpet and mussel will also allow the comparison with
588 worldwide biomonitoring surveys that mainly use *Mytilus* sp.

589 The results of the present study show that the water contamination in North Corsica port areas
590 is relatively low compared to other ports areas in the Mediterranean Sea. For future research, it
591 is necessary to continue this monitoring over time and at different seasons to obtain more
592 reliable data.

593 Finally, the validation of the sampling techniques as “pool” is important for management
594 purpose. Even if the “pooled” data limits the analyses for fundamental research, this method
595 gives reliable results to help the local managers and authorities for planning integrated
596 environmental management of the port area.

597

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783

784 **Statements and declarations**

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790

791 **Competing Interests**

792 The authors have no relevant financial or non-financial interests to disclose.

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794

795 **Authors Contributions**

796 Marion Pillet: conceptualization, methodology, validation, data collection, formal analyses,
797 investigation, supervision, writing.

798 Mathilde Dabrowski: methodology, data collection, formal analyses.

799 Michel Marengo: conceptualization, methodology, validation, data collection, supervision,
800 project administration, funding acquisition

801 Lovina Fullgrabe: writing

802 Michèle Leduc: methodology, data collection.

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806 Carine Churlaud: formal analyses.

807 Pierre Lejeune: conceptualization, funding acquisition.

808 Hélène Thomas: supervision, project administration, funding acquisition.

809

810 **Ethical Approval**

811 This study does not involve human participants or vertebrate animals.

812

813 **Consent to Participate**

814 Yes

815

816 **Consent to Publish**

817 Yes

818

819 **Availability of data and materials**

820 The datasets generated during and/or analysed during the current study are available from the

821 corresponding author on reasonable request.

822

823 List of captions:

824 **Fig. 3** Map of the experimental locations in North Corsica (France) and anthropic pressures
825 observed next to the different ports

826 **Fig. 4** Occurrence of the different class of difference (in %) between « pool » and « individual »
827 techniques, for biomarker of effect and exposure (trace element and organic pollutant
828 bioaccumulation) in the three marine molluscs

829 **Fig. 3** PCA results for trace element and organic contaminants in (A) sea cucumber and (B)
830 limpet tissues per location

831 **Fig. 4** Total enzymatic activity of superoxide dismutase (U/g of wet tissue) and glutathione S-
832 transferase (mU/g of wet tissue) in digestive gland of mussel, soft tissue of limpet and body
833 wall of sea cucumber collected in North Corsica in January (mean \pm standard deviation).
834 Different letters indicate significant difference between the location, within the same specie (P
835 < 0.05). Circles indicate the value for the pooled data collected in the same location

836 **Fig. 5** Total enzymatic activity of lactate dehydrogenase, pyruvate kinase and
837 phosphoenolpyruvate carboxykinase (mU/g of wet tissue) in digestive gland of mussel, soft
838 tissue of limpet and body wall of sea cucumber collected in North Corsica in January (mean \pm
839 standard deviation). Different letters indicate significant difference between the location, within
840 the same specie ($P < 0.05$). Circles indicate the value for the pooled data collected in the same
841 location

842 **Fig. 6** Total enzymatic activity of acetylcholinesterase and laccase (mU/g of wet tissue) in
843 digestive gland of mussel, soft tissue of limpet and body wall of sea cucumber collected in
844 North Corsica in January (mean \pm standard deviation). Different letters indicate significant
845 difference between the location, within the same specie ($P < 0.05$). Circles indicate the value
846 for the pooled data collected in the same location

847 **Fig. 7** Total lipids and glycogen concentrations (mg/g of wet tissues) in digestive gland of
848 mussel, soft tissue of limpet and body wall of sea cucumber collected in North Corsica in
849 January (mean \pm standard deviation). Different letters indicate significant difference between
850 the location, within the same specie ($P < 0.05$). Circles indicate the value for the pooled data
851 collected in the same location

852 **Fig. 8** Integrated Biomarker Responses (IBR, mean) calculated for limpet and sea cucumber
853 using the biomarker of effects. Different letters indicate significant difference between the
854 location, within the same specie ($P < 0.05$)