



**HAL**  
open science

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

Marion Pillet, Mathilde Dabrowski, Michel Marengo, Lovina Fullgrabe, Michèle Leduc, Quentin Fontaine, Stéphane Le Floch, Valérie Huet, Carine Churlaud, Pierre Lejeune, et al.

### ► **To cite this version:**

Marion Pillet, Mathilde Dabrowski, Michel Marengo, Lovina Fullgrabe, Michèle Leduc, et al.. Preliminary inter-port study of the quality of environments using physiological responses of invertebrates exposed to chronic trace element and organic contamination in Corsica (Mediterranean Sea). *Ecotoxicology*, 2023, 32 (2), pp.243-260. 10.1007/s10646-023-02635-w . hal-04019013

**HAL Id: hal-04019013**

**<https://hal.science/hal-04019013>**

Submitted on 30 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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)**

4  
5 Marion Pillet<sup>1,2\*</sup> (Orcid : 0000-0002-4486-595X), Mathilde Dabrowski<sup>1</sup>, Michel Marengo<sup>2</sup>  
6 (Orcid : 0000-0001-8419-5076), Lovina Fullgrabe<sup>2</sup> (Orcid: 0000-0003-3798-4253), Michèle  
7 Leduc<sup>2</sup> (Orcid : 0000-0001-7086-5999), Quentin Fontaine (Orcid: 0000-0003-0175-5889),  
8 Stéphane Le Floch<sup>3</sup> (Orcid: 0000-0002-5794-7284), Valérie Huet<sup>1</sup> (Orcid: 0000-0003-0976-  
9 5600 ), Carine Churlaud<sup>1</sup> (Orcid: 0000-0002-1838-3252), Pierre Lejeune<sup>2</sup> (Orcid: 0000-0002-  
10 2419-4896) & Hélène Thomas<sup>1</sup> (Orcid: 0000-0002-7932-6841)

11  
12 <sup>1</sup>Littoral ENvironnement et Sociétés (UMR7266), La Rochelle Université, 2 rue Olympe de  
13 Gouges, 17000 La Rochelle, France

14 <sup>2</sup>STation de REcherches Sous-marines et Océanographiques, Punta Revellata, BP33, 20260  
15 Calvi, France

16 <sup>3</sup>Centre de Documentation, de Recherche et d'Expérimentations sur les Pollutions Accidentelles  
17 des Eaux, 715 rue Alain Colas, CS 41836, 29218 Brest Cedex 2, France

18  
19 \*Corresponding author at: [marion.pillet@univ-lr.fr](mailto:marion.pillet@univ-lr.fr)

20  
21 **Acknowledgment**

22 The authors are grateful to Mr. B. Pantalacci, manager of the shellfish farm *SARL Etang de*  
23 *Diane*, and his employees who provided mussels.

24  
25 **Funding**

26 This work was supported by the “QUALity of the marine environment in the Mediterranean  
27 POrt areas” (QUAMPO) project, funded by the “Fonds Européen pour les Affaires Maritimes  
28 et la Pêche” (FEAMP) - European Maritime and Fisheries Fund (EMFF) [grant number  
29 PFEA800219DM0940001, 2019].

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 Mohammadzadeh 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.

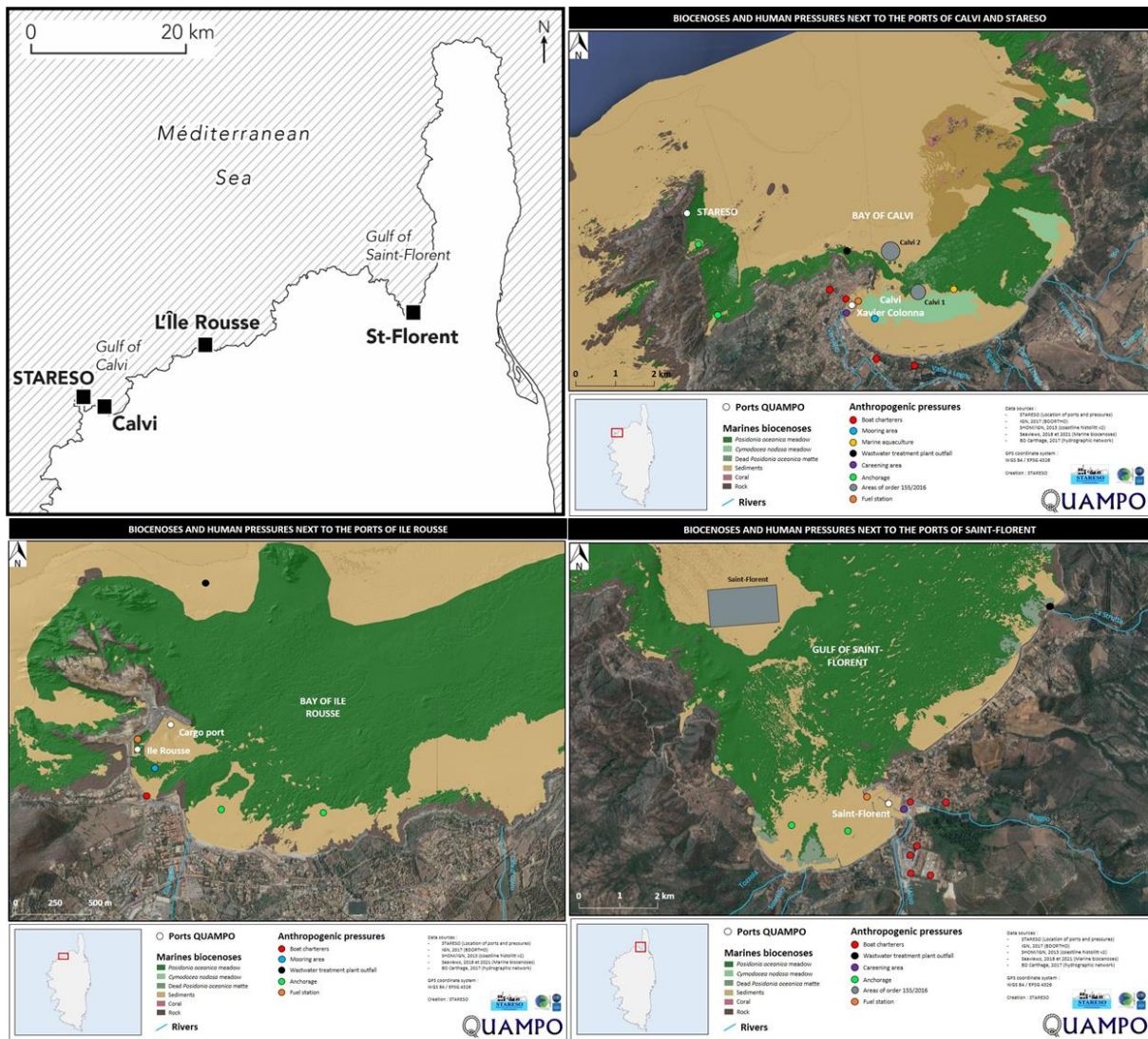
142

### 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 =  $76.75 \pm 9.03$   
165 mm) were found only in St-Florent port. Limpets (n = 13-14 per location; length =  $35.24 \pm 5.36$   
166 mm) were found everywhere except in St-Florent port. Sea cucumbers (n = 5-7 per location;  
167 length =  $154.1 \pm 43.77$  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 =  $68.56 \pm 8.84$  mm) and four sea cucumbers (length  
170 =  $193.25 \pm 34.67$  mm) were collected in St-Florent and 14 limpets (length =  $32.34 \pm 4.93$  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% HNO<sub>3</sub>/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<sup>-1</sup> 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<sup>-1</sup>  
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<sup>-1</sup> 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<sup>®</sup> (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. $\text{mg}^{-1}$  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)).

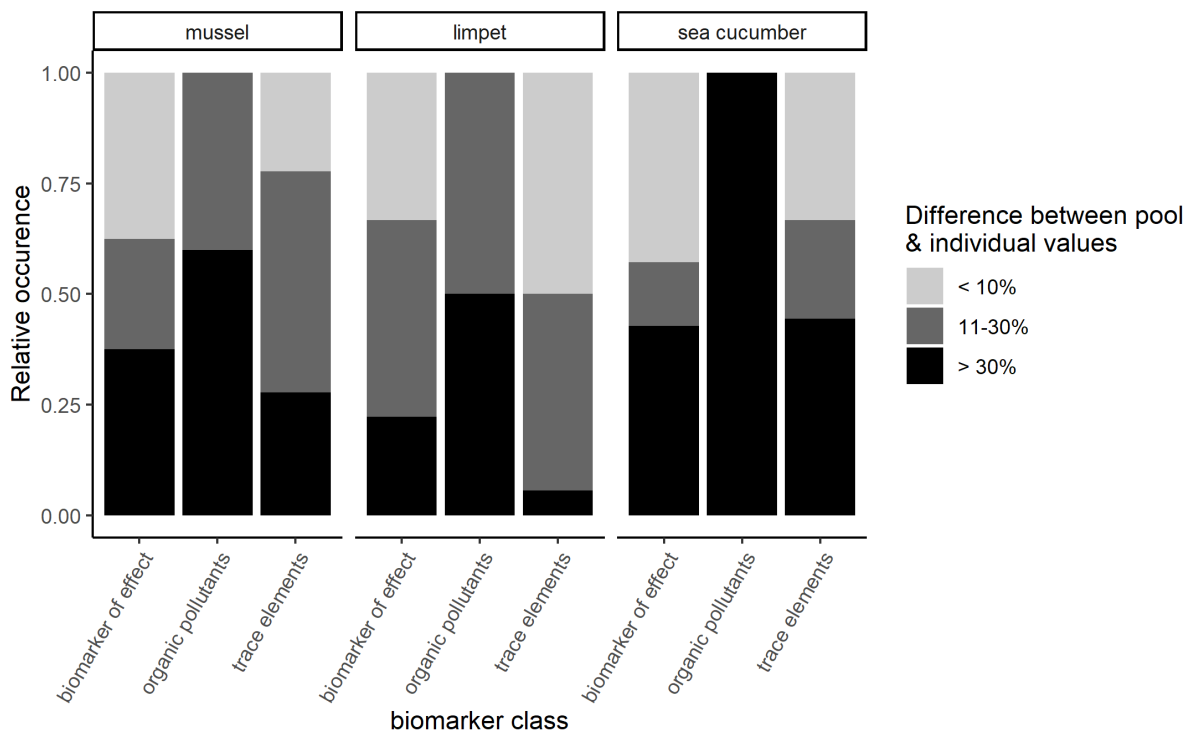
306

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 <sup>b</sup>	382.54 $\pm$ 150.34 <sup>a</sup>	497.18 $\pm$ 186.09 <sup>a</sup>	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 <sup>ab</sup>	16.21 $\pm$ 2.02 <sup>b</sup>	41.28 $\pm$ 11.53 <sup>a</sup>	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 <sup>b</sup>	1.78 $\pm$ 1.01 <sup>ab</sup>	4.48 $\pm$ 4.34 <sup>a</sup>	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 <sup>b</sup>	0.39 $\pm$ 0.08 <sup>a</sup>	0.33 $\pm$ 0.1 <sup>a</sup>	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 <sup>a</sup>	10.09 $\pm$ 1.92 <sup>a</sup>	2.42 $\pm$ 0.94 <sup>b</sup>	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 <sup>b</sup>	110.18 $\pm$ 21.6 <sup>a</sup>	55.93 $\pm$ 28.39 <sup>a</sup>	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 <sup>b</sup>	2091.53 $\pm$ 405.59 <sup>a</sup>	2041.55 $\pm$ 708.44 <sup>a</sup>	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 <sup>b</sup>	6.21 $\pm$ 1.8 <sup>a</sup>	6.08 $\pm$ 1.65 <sup>a</sup>	5.26	0.78 $\pm$ 0.3 <sup>a</sup>	1.64 $\pm$ 0.77 <sup>ab</sup>	1.14 $\pm$ 0.7 <sup>a</sup>	29.11 $\pm$ 55.3 <sup>b</sup>	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 <sup>a</sup>	5.97 $\pm$ 1.27 <sup>a</sup>	1.95 $\pm$ 0.49 <sup>b</sup>	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 <sup>b</sup>	4.36 $\pm$ 1.03 <sup>ab</sup>	7.04 $\pm$ 2.71 <sup>a</sup>	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 <sup>b</sup>	1.46 $\pm$ 0.08 <sup>a</sup>	1.43 $\pm$ 0.15 <sup>a</sup>	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 <sup>b</sup>	5.43 $\pm$ 2.65 <sup>a</sup>	2.6 $\pm$ 0.77 <sup>b</sup>	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 <sup>c</sup>	218.88 $\pm$ 22.82 <sup>a</sup>	132.84 $\pm$ 21.11 <sup>b</sup>	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

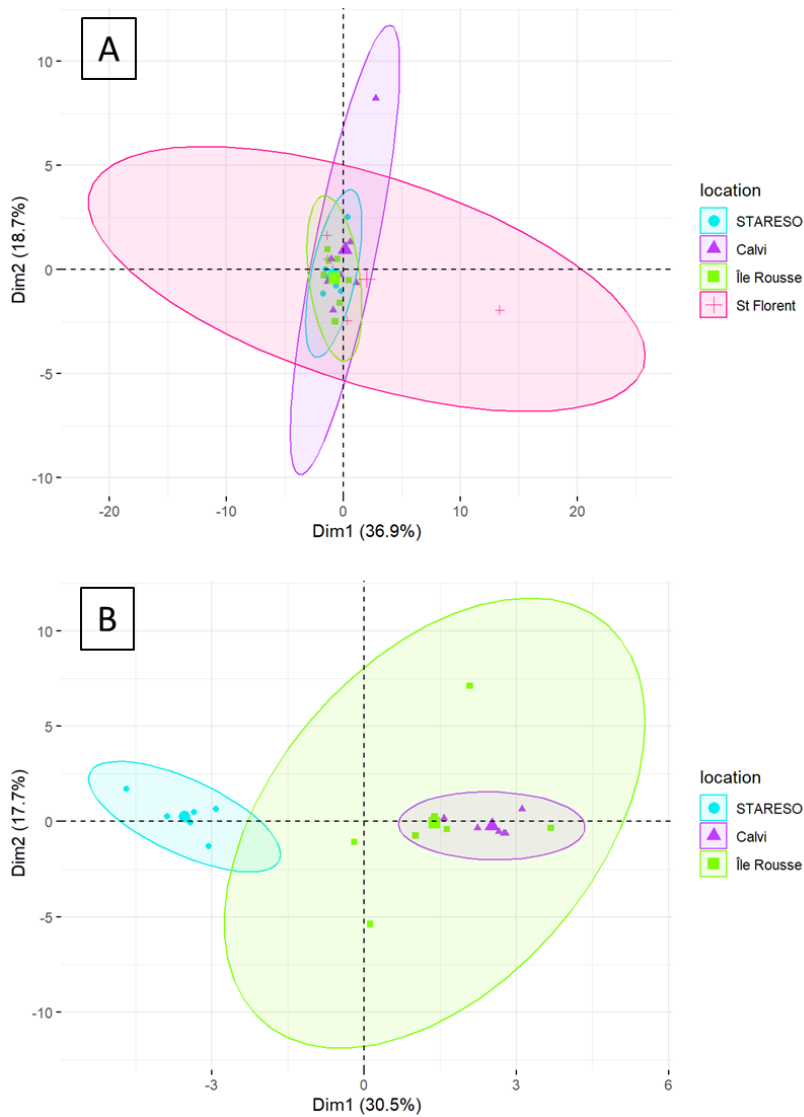
PCB 7	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 28	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 52	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 35	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 101	2.80	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
PCB 77	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD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

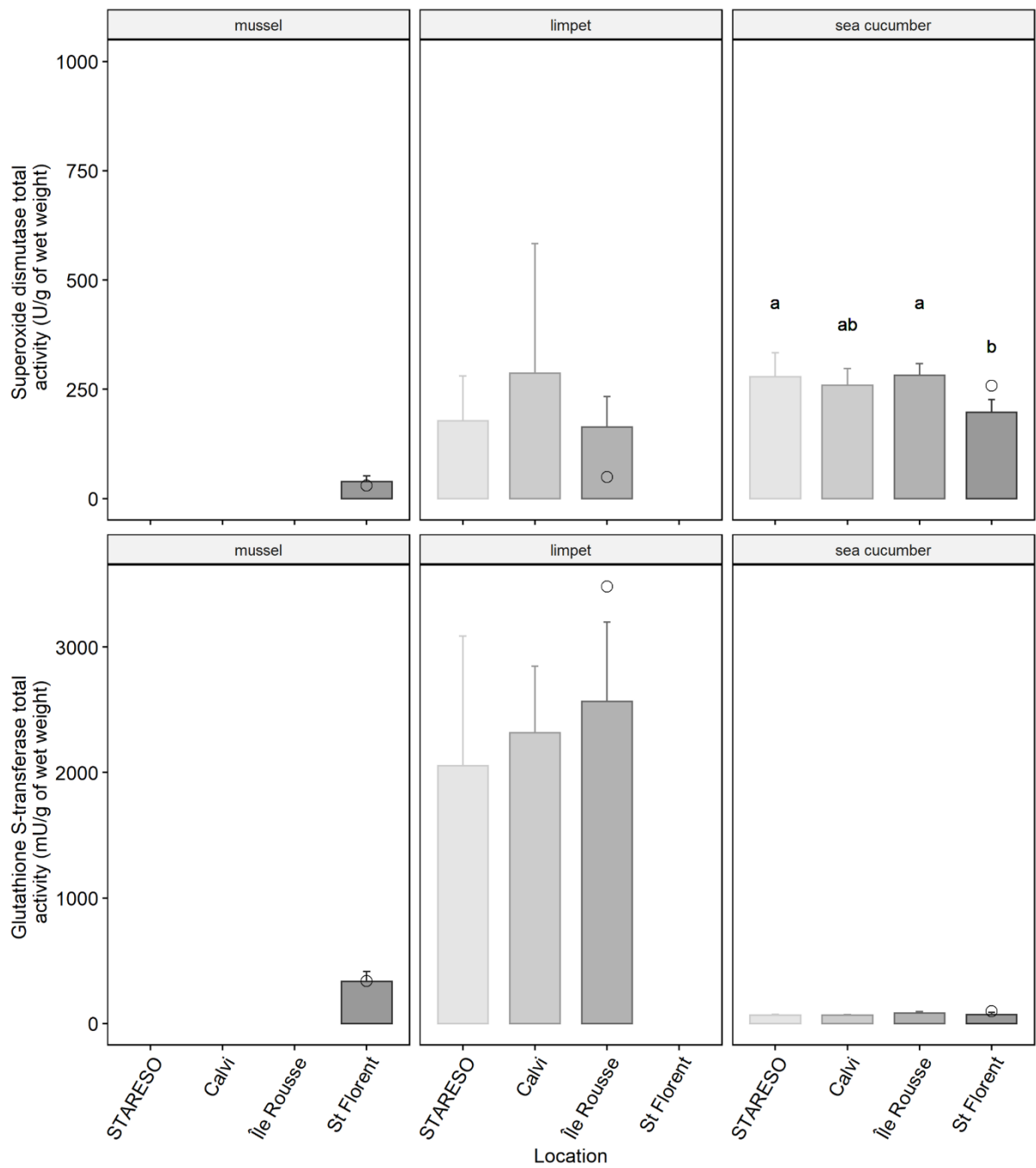
385

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

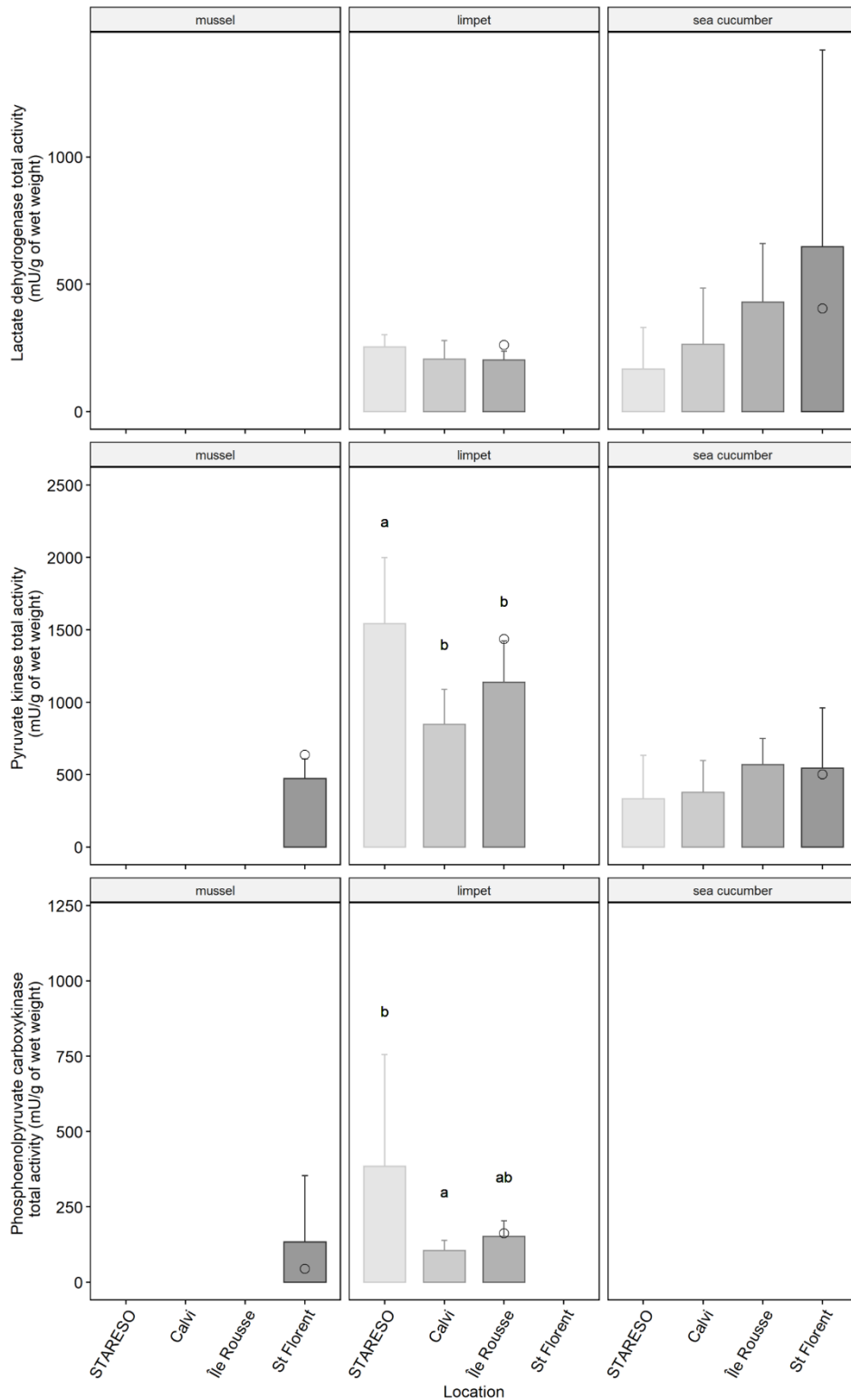
391 The response of the antioxidant system varied according to the species (Figure 4). In limpet the  
 392 SOD and GST activities were much higher than those detected in mussel (and in sea cucumber  
 393 for GST). The effect of location was significant for sea cucumber SOD activity. Its SOD activity  
 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

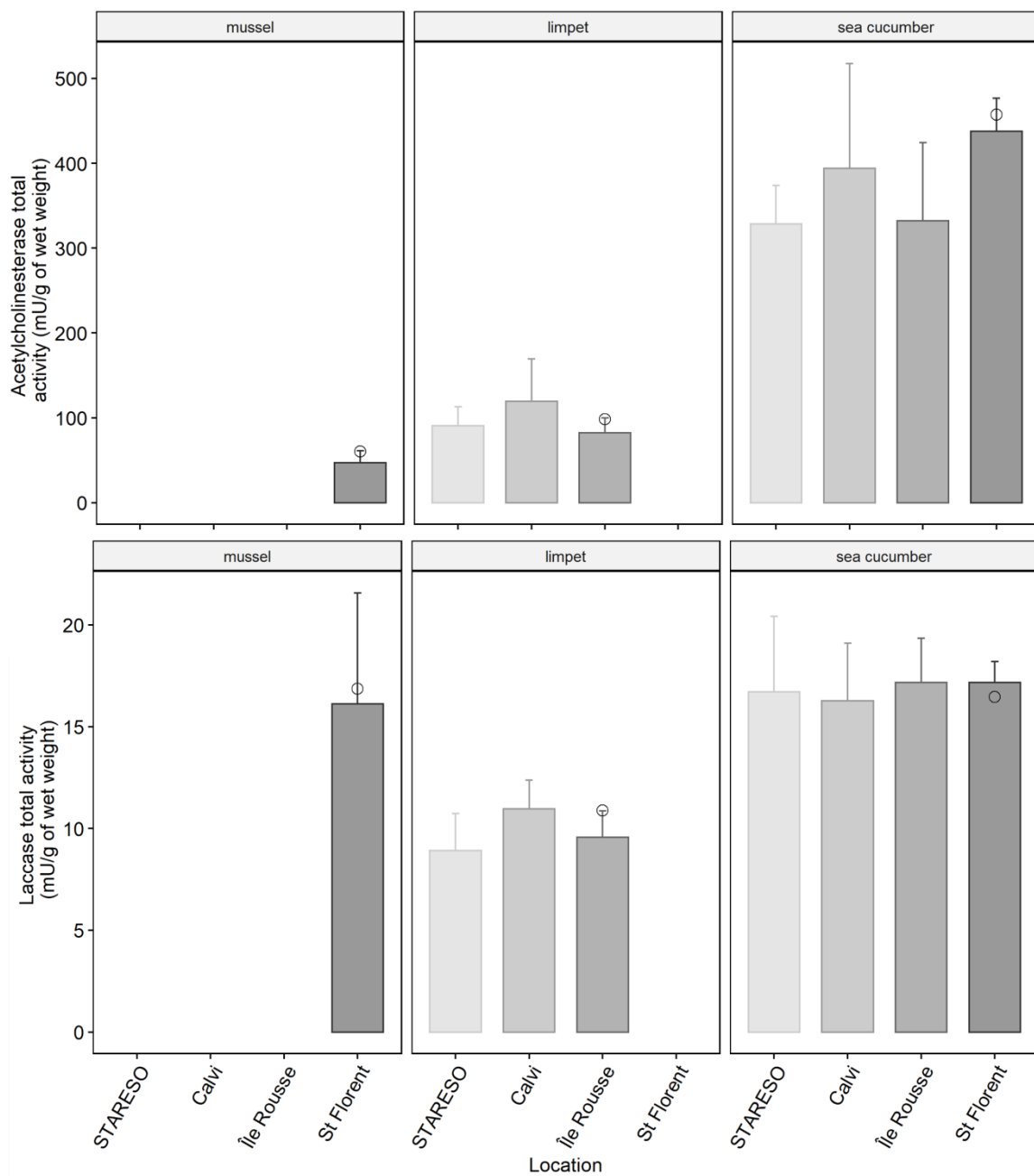
**Fig. 5** Total enzymatic activity of lactate dehydrogenase, pyruvate kinase and phosphoenolpyruvate carboxykinase (mU/g of wet tissue) in digestive gland of mussel, soft tissue of limpet and body wall of sea cucumber collected in North Corsica in January (mean ± standard deviation). Different letters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



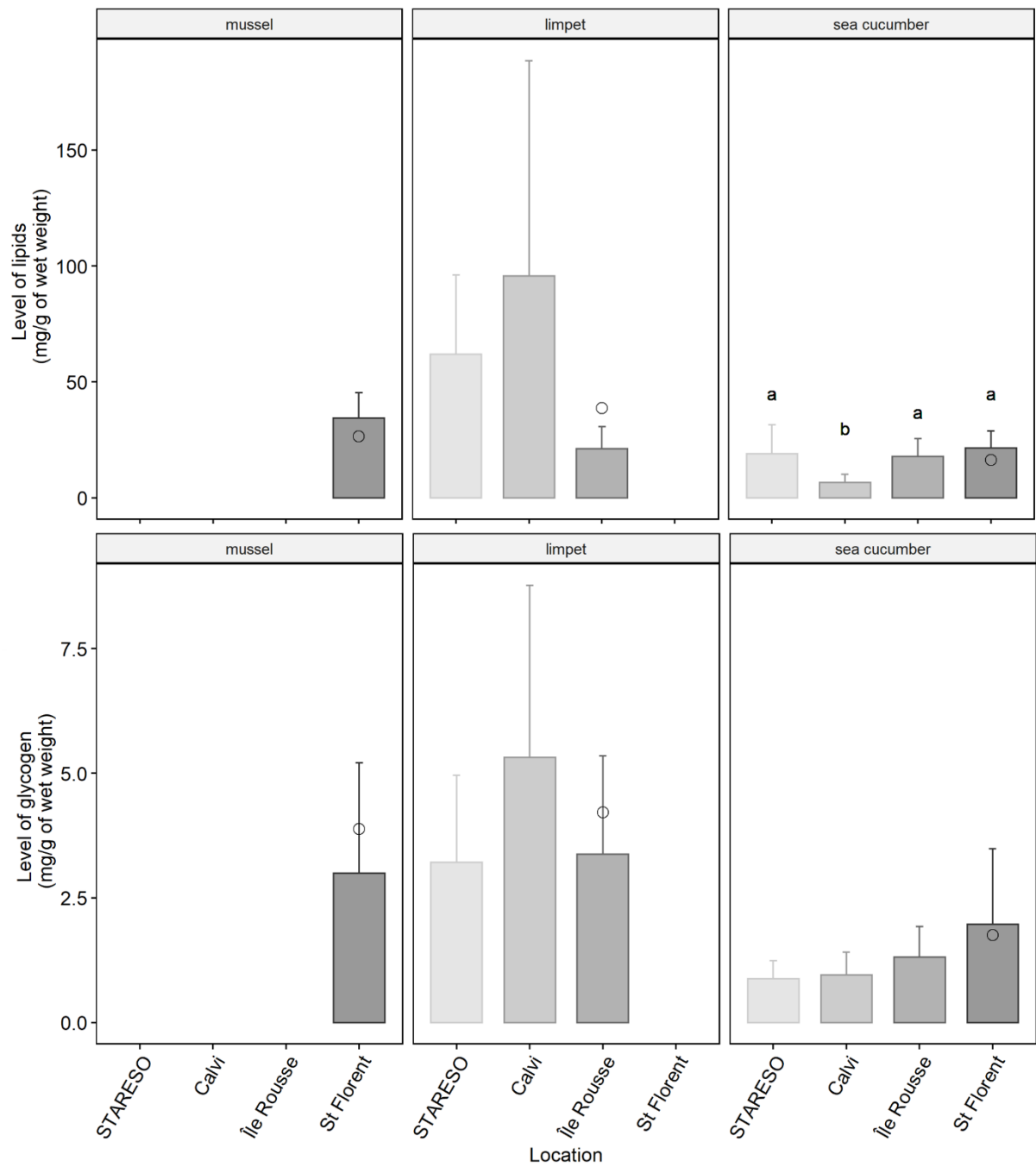
422

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

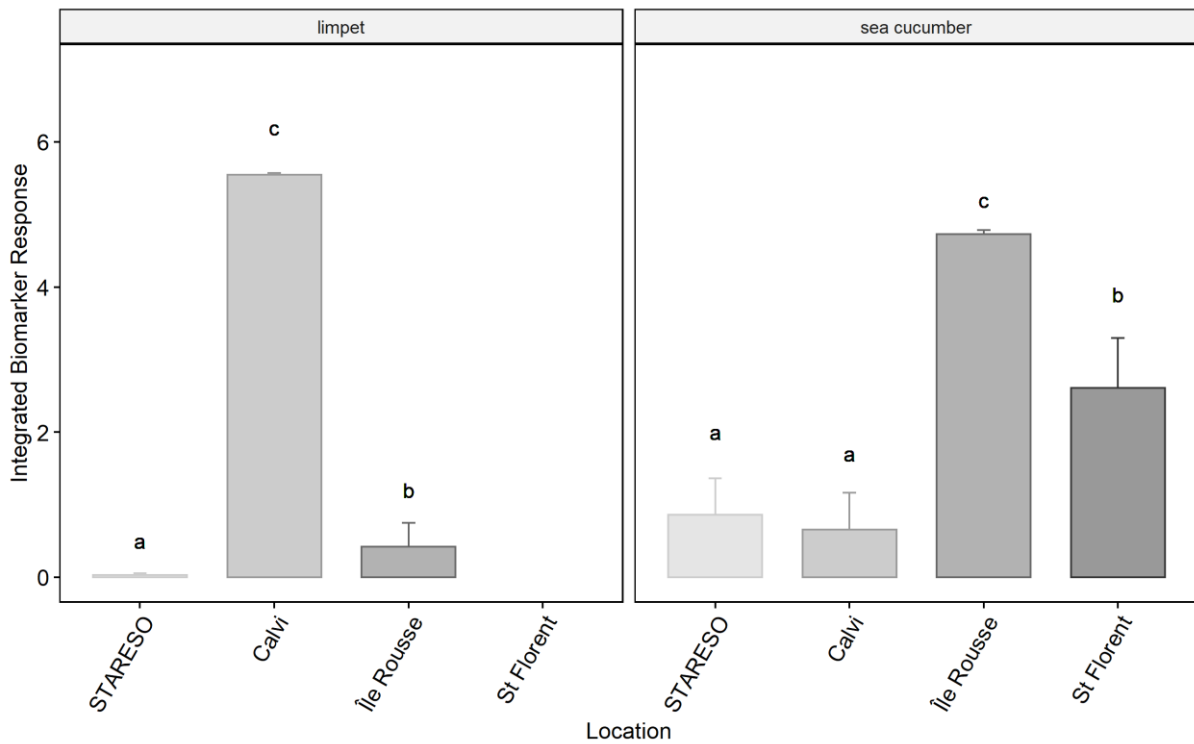
435 **Fig. 7** Total lipids and glycogen concentrations (mg/g of wet tissues) in digestive gland of  
 436 mussel, soft tissue of limpet and body wall of sea cucumber collected in North Corsica in  
 437 January (mean  $\pm$  standard deviation). Different letters indicate significant difference between  
 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 µg/g for Cd, 2.43 µg/g  
515 for Cu, 2.09 µg/g for Pb, 14.58 µg/g for Ni, 16.86 µg/g for Zn, and 73.46 µg/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 µg/g) and higher for Cu, Zn and Fe (respectively, 4.8; 41.67 and 126.45 µg/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

- 599 Andral B, Stanisiere JY, Sauzade D, Damier E, Thebault H, Galgani F, Boissery P (2004)  
600 Monitoring chemical contamination levels in the Mediterranean based on the use of mussel  
601 caging. *Marine Pollution Bulletin* 49: 704-712.
- 602 Aydin M, Tunca E, Alver Şahin Ü (2017) Effects of anthropological factors on the metal  
603 accumulation profiles of sea cucumbers in near industrial and residential coastlines of İzmir,  
604 Turkey. *International Journal of Environmental Analytical Chemistry* 97: 368-382.
- 605 Bailey DM, Genard B, Collins MA, Rees J-F, Unsworth SK, Battle EJV, Bagley PM, Jamieson  
606 AJ, Priede IG (2005) High swimming and metabolic activity in the deep-sea eel  
607 *Synaphobranchus kaupii* revealed by integrated in situ and in vitro measurements.  
608 *Physiological and Biochemical Zoology* 78: 335-346.
- 609 Beg MU, Butt SA, Al-Dufaileej S, Karam Q, Al-Sharrah TK, Saeed T (2018) Biomarkers in  
610 fish as a measure of the state of marine environment of Kuwait. *Environmental Monitoring and*  
611 *Assessment* 190: 325.
- 612 Beliaeff B, Burgeot T (2002) Integrated biomarker response: A useful tool for ecological risk  
613 assessment. *Environmental Toxicology and Chemistry* 21: 1316-1322.
- 614 Benali I, Boutiba Z, Merabet A, Chèvre N (2015) Integrated use of biomarkers and condition  
615 indices in mussels (*Mytilus galloprovincialis*) for monitoring pollution and development of  
616 biomarker index to assess the potential toxic of coastal sites. *Marine Pollution Bulletin* 95: 385-  
617 394.
- 618 Bertrand L, Monferrán MV, Mouneyrac C, Amé MV (2018) Native crustacean species as a  
619 bioindicator of freshwater ecosystem pollution: A multivariate and integrative study of multi-  
620 biomarker response in active river monitoring. *Chemosphere* 206: 265-277.
- 621 Bignert A, Göthberg A, Jensen S, Litzén K, Odsjö T, Olsson M, Reutergårdh L (1993) The  
622 need for adequate biological sampling in ecotoxicological investigations: a retrospective study  
623 of twenty years pollution monitoring. *Science of the Total Environment* 128: 121-139.
- 624 Bignert A, Olsson M, de Wit C, Litzén K, Rappe C, Reutergårdh L (1994) Biological variation  
625 — an important factor to consider in ecotoxicological studies based on environmental samples.  
626 *Fresenius' Journal of Analytical Chemistry* 348: 76-85.
- 627 Breitwieser M, Dubillot E, Barbarin M, Churlaud C, Huet V, Muttin F, Thomas H (2018)  
628 Assessment of the biological quality of port areas: A case study on the three harbours of La  
629 Rochelle: The marina, the fishing harbour and the seaport. *PLOS ONE* 13: e0198255.
- 630 Campanella L, Conti ME, Cubadda F, Sucapane C (2001) Trace metals in seagrass, algae and  
631 molluscs from an uncontaminated area in the Mediterranean. *Environmental Pollution* 111:  
632 117-126.
- 633 Cao R, Zhang T, Li X, Zhao Y, Wang Q, Yang D, Qu Y, Liu H, Dong Z, Zhao J (2019) Seawater  
634 acidification increases copper toxicity: A multi-biomarker approach with a key marine  
635 invertebrate, the Pacific Oyster *Crassostrea gigas*. *Aquatic Toxicology* 210: 167-178.
- 636 Caricato R, Lionetto MG, Schettino T (2010) Seasonal variation of biomarkers in *Mytilus*  
637 *galloprovincialis* sampled inside and outside Mar Piccolo of Taranto (Italy). *Chemistry and*  
638 *Ecology* 26: 143-153.
- 639 Carr RS, Neff JM (1984) Quantitative semi-automated enzymatic assay for tissue glycogen.  
640 *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 77: 447-449.
- 641 Childress JJ, Somero GN (1979) Depth-related enzymic activities in muscle, brain and heart of  
642 deep-living pelagic marine teleosts. *Marine Biology* 52: 273-283.
- 643 Conti ME, Mele G, Finioia MG (2017) Baseline trace metals in *Patella caerulea* in a central  
644 Tyrrhenian ecosystem (Pontine Islands archipelago and Lazio region coastal sites, Italy).  
645 *Environmental Science and Pollution Research* 24: 8852-8865.

646 Cravo A, Lopes B, Serafim Â, Company R, Barreira L, Gomes T, Bebianno MJ (2009) A  
647 multibiomarker approach in *Mytilus galloprovincialis* to assess environmental quality. Journal  
648 of Environmental Monitoring 11: 1673-1686.

649 Cravo A, Pereira C, Gomes T, Cardoso C, Serafim A, Almeida C, Rocha T, Lopes B, Company  
650 R, Medeiros A, Norberto R, Pereira R, Araújo O, Bebianno MJ (2012) A multibiomarker  
651 approach in the clam *Ruditapes decussatus* to assess the impact of pollution in the Ria Formosa  
652 lagoon, South Coast of Portugal. Marine Environmental Research 75: 23-34.

653 DCSMM (2008) Directive 2008/56/EC of 17 June 2008 establishing a framework for  
654 community action in the field of marine environmental policy (Marine Strategy Framework  
655 Directive), Official Journal of the European Union. European Commission.

656 Delahaut V, Daelemans O, Sinha AK, De Boeck G, Bervoets L (2019) A multibiomarker  
657 approach for evaluating environmental contamination: common carp (*Cyprinus carpio*)  
658 transplanted along a gradient of metal pollution. Science of the Total Environment 669: 481-  
659 492.

660 Devin S, Burgeot T, Giambérini L, Minguez L, Pain-Devin S (2014) The integrated biomarker  
661 response revisited: optimization to avoid misuse. Environmental Science and Pollution  
662 Research 21: 2448-2454.

663 Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric  
664 determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88-95.

665 Fasulo S, Iacono F, Cappello T, Corsaro C, Maisano M, D'Agata A, Giannetto A, De Domenico  
666 E, Parrino V, Lo Paro G, Mauceri A (2012) Metabolomic investigation of *Mytilus*  
667 *galloprovincialis* (Lamarck 1819) caged in aquatic environments. Ecotoxicology and  
668 Environmental Safety 84: 139-146.

669 Frings CS, Fendley TW, Dunn RT, Queen CA (1972) Improved Determination of Total Serum  
670 Lipids by the Sulfo-Phospho-Vanillin Reaction. Clinical Chemistry 18: 673-675.

671 Galgani F, Chiffolleau JF, Orsoni V, Costantini L, Boissery P, Calendini S, Andral B (2006)  
672 Chemical contamination and sediment toxicity along the coast of Corsica. Chemistry and  
673 Ecology 22: 299-312.

674 Gharred T, Helaoui A, Mannai R, Jebali J (2019) Effect of multiple pollution on the  
675 cholinesterase activity, morphometry and reproduction performance of *Patella caerulea*  
676 collected from eastern Tunisian coasts. Cahiers de Biologie Marine 60: 11-20.

677 Goldberg ED (1975) The mussel watch — A first step in global marine monitoring. Marine  
678 Pollution Bulletin 6: 111.

679 Gouveia N, Oliveira CRM, Martins CP, Maranhão LA, Seabra Pereira CD, de Orte MR,  
680 Harayashiki CAY, Almeida SM, Castro IB (2019) Can shell alterations in limpets be used as  
681 alternative biomarkers of coastal contamination? Chemosphere 224: 9-19.

682 Guerra-García JM, Navarro-Barranco C, Martínez-Laiz G, Moreira J, Giráldez I, Morales E,  
683 Fernández-Romero A, Florido M, Ros M (2021) Assessing environmental pollution levels in  
684 marinas. Science of the Total Environment 762: 144169.

685 Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step  
686 in mercapturic acid formation. Journal of Biological Chemistry 249: 7130-7139.

687 Islam SM, Tanaka M (2004) Impacts of pollution on coastal and marine ecosystems including  
688 coastal and marine fisheries and approach for management: a review and synthesis. Marine  
689 Pollution Bulletin 48: 624-649.

690 Jamieson PM, Nyirenda MJ, Walker BR, Chapman KE, Seckl JR (1999) Interactions between  
691 oestradiol and glucocorticoid regulatory effects on liver-specific glucocorticoid-inducible  
692 genes: possible evidence for a role of hepatic 11 $\beta$ -hydroxysteroid dehydrogenase type 1. Journal  
693 of Endocrinology 160: 103-109.

694 Ji R, Pan L, Guo R, Zheng L, Zhang M (2019) Using multi-integrated biomarker indexes  
695 approach to assess marine quality and health status of marine organism: a case study of

696 *Ruditapes philippinarum* in Laizhou Bay, China. Environmental Science and Pollution  
697 Research 26: 9916-9930.

698 Lacroix C, Le Cuff N, Receveur J, Moraga D, Auffret M, Guyomarch J (2014) Development  
699 of an innovative and “green” stir bar sorptive extraction–thermal desorption–gas  
700 chromatography–tandem mass spectrometry method for quantification of polycyclic aromatic  
701 hydrocarbons in marine biota. Journal of Chromatography A 1349: 1-10.

702 Lafabrie C, Pergent-Martini C, Pergent G (2008) Metal contamination of *Posidonia oceanica*  
703 meadows along the Corsican coastline (Mediterranean). Environmental Pollution 151: 262-268.

704 Lafabrie C, Pergent G, Kantin R, Pergent-Martini C, Gonzalez JL (2007) Trace metals  
705 assessment in water, sediment, mussel and seagrass species – Validation of the use of *Posidonia*  
706 *oceanica* as a metal biomonitor. Chemosphere 68: 2033-2039.

707 Lehtonen KK, d'Errico G, Korpinen S, Regoli F, Ahkola H, Kinnunen T, Lastumäki A (2019)  
708 Mussel Caging and the Weight of Evidence Approach in the Assessment of Chemical  
709 Contamination in Coastal Waters of Finland (Baltic Sea). Frontiers in Marine Science 6: 688.

710 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin  
711 phenol reagent. Journal of Biological Chemistry 193: 265-275.

712 Luna-Acosta A, Breitwieser M, Renault T, Thomas-Guyon H (2017a) Recent findings on  
713 phenoloxidases in bivalves. Marine Pollution Bulletin 122: 5-16.

714 Luna-Acosta A, Bustamante P, Budzinski H, Huet V, Thomas-Guyon H (2015) Persistent  
715 organic pollutants in a marine bivalve on the Marennes-Oléron Bay and the Gironde Estuary  
716 (French Atlantic Coast) - Part 2: Potential biological effects. Science of the Total Environment  
717 514: 511-522.

718 Luna-Acosta A, Bustamante P, Thomas-Guyon H, Zaldibar B, Izagirre U, Marigómez I (2017b)  
719 Integrative biomarker assessment of the effects of chemically and mechanically dispersed crude  
720 oil in Pacific oysters, *Crassostrea gigas*. Science of the Total Environment 598: 713-721.

721 Luna-Acosta A, Rosenfeld E, Amari M, Fruitier-Arnaudin I, Bustamante P, Thomas-Guyon H  
722 (2010) First evidence of laccase activity in the Pacific oyster *Crassostrea gigas*. Fish &  
723 Shellfish Immunology 28: 719-726.

724 Maisano M, Cappello T, Natalotto A, Vitale V, Parrino V, Giannetto A, Oliva S, Mancini G,  
725 Cappello S, Mauceri A, Fasulo S (2017) Effects of petrochemical contamination on caged  
726 marine mussels using a multi-biomarker approach: Histological changes, neurotoxicity and  
727 hypoxic stress. Marine Environmental Research 128: 114-123.

728 Mbandzi N, Vincent Nakin MD, Oyedeji AO (2022) Temporal and spatial variation of heavy  
729 metal concentration in four limpet species along the southeast coast of South Africa.  
730 Environmental Pollution 302: 119056.

731 Mohammadzadeh M, Bastami K, Ehsanpour M, Afkhami M, Mohammadzadeh F,  
732 Esmailzadeh M (2016) Heavy metal accumulation in tissues of two sea cucumbers, *Holothuria*  
733 *leucospilota* and *Holothuria scabra* in the northern part of Qeshm Island, Persian Gulf. Marine  
734 Pollution Bulletin 103.

735 Orani AM, Barats A, Vassileva E, Thomas OP (2018) Marine sponges as a powerful tool for  
736 trace elements biomonitoring studies in coastal environment. Marine Pollution Bulletin 131:  
737 633-645.

738 Paoletti F, Aldinucci D, Mocali A, Caparrini A (1986) A sensitive spectrophotometric method  
739 for the determination of superoxide dismutase activity in tissue extracts. Analytical  
740 Biochemistry 154: 536-541.

741 Parra-Luna M, Martín-Pozo L, Hidalgo F, Zafra-Gómez A (2020) Common sea urchin  
742 (*Paracentrotus lividus*) and sea cucumber of the genus *Holothuria* as bioindicators of pollution  
743 in the study of chemical contaminants in aquatic media. A revision. Ecological Indicators 113:  
744 106185.

745 Pérez S, Sánchez-Marín P, Bellas J, Viñas L, Besada V, Fernández N (2019) Limpets (*Patella*  
746 spp. Mollusca, Gastropoda) as model organisms for biomonitoring environmental quality.  
747 Ecological Indicators 101: 150-162.

748 Petrescu I, Bojan O, Saied M, Bârză O, Schmidt F, Kühnle HF (1979) Determination of  
749 phosphoenolpyruvate carboxykinase activity with deoxyguanosine 5'-diphosphate as  
750 nucleotide substrate. Analytical Biochemistry 96: 279-281.

751 Rainbow PS (2002) Trace metal concentrations in aquatic invertebrates: why and so what?  
752 Environmental Pollution 120: 497-507.

753 Rainbow PS, Phillips DJH (1993) Cosmopolitan biomonitors of trace metals. Marine Pollution  
754 Bulletin 26: 593-601.

755 Reguera P, Couceiro L, Fernández N (2018) A review of the empirical literature on the use of  
756 limpets *Patella* spp. (Mollusca: Gastropoda) as bioindicators of environmental quality.  
757 Ecotoxicology and Environmental Safety 148: 593-600.

758 Richir J, Gobert S (2014) A reassessment of the use of *Posidonia oceanica* and *Mytilus*  
759 *galloprovincialis* to biomonitor the coastal pollution of trace elements: New tools and tips.  
760 Marine Pollution Bulletin 89: 390-406.

761 Serafim A, Company R, Lopes B, Fonseca VF, França S, Vasconcelos RP, Bebianno MJ,  
762 Cabral HN (2012) Application of an integrated biomarker response index (IBR) to assess  
763 temporal variation of environmental quality in two Portuguese aquatic systems. Ecological  
764 Indicators 19: 215-225.

765 Shrivastava A, Gupta V (2011) Methods for the determination of limit of detection and limit of  
766 quantitation of the analytical methods. Chronicles of Young Scientists 2: 21-25.

767 Silva CSE, Novais SC, Simões T, Caramalho M, Gravato C, Rodrigues MJ, Maranhão P, Lemos  
768 MFL (2018) Using biomarkers to address the impacts of pollution on limpets (*Patella depressa*)  
769 and their mechanisms to cope with stress. Ecological Indicators 95: 1077-1086.

770 Turk Culha S, Dereli H, Karaduman FR, Culha M (2016) Assessment of trace metal  
771 contamination in the sea cucumber (*Holothuria tubulosa*) and sediments from the Dardanelles  
772 Strait (Turkey). Environmental Science and Pollution Research 23: 11584-11597.

773 Turle R, Collins B (1992) Validation of the use of pooled samples for monitoring of  
774 contaminants in wildlife. Chemosphere 25: 463-469.

775 Uluturhan E, Darılmaz E, Kontas A, Bilgin M, Alyuruk H, Altay O, Sevgi S (2019) Seasonal  
776 variations of multi-biomarker responses to metals and pesticides pollution in *M.*  
777 *galloprovincialis* and *T. decussatus* from Homa Lagoon, Eastern Aegean Sea. Marine Pollution  
778 Bulletin 141: 176-186.

779 Wang WX (2016) Chapter 4 - Bioaccumulation and Biomonitoring, in: Blasco J, Chapman PM,  
780 Campana O, Hampel M (Eds.), Marine Ecotoxicology. Academic Press, pp. 99-119.

781

782

783



784 **Statements and declarations**

785 **Funding**

786 This work was supported by the “QUALity of the marine environment in the Mediterranean  
787 POrt areas” (QUAMPO) project, funded by the “Fonds Européen pour les Affaires Maritimes  
788 et la Pêche” (FEAMP) - European Maritime and Fisheries Fund (EMFF) [grant number  
789 PFEA800219DM0940001, 2019].

790

791 **Competing Interests**

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

793 The authors have no competing interests to declare that are relevant to the content of this article.

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.

803 Quentin Fontaine: formal analyses.

804 Stéphane Le Floch: methodology, formal analyses.

805 Valérie Huet: formal analyses.

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 ( $P < 0.05$ )