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1 **First characterisation of seasonal variations in biomarkers baseline in *Patella* sp. from**
2 **Mediterranean ports (North Corsica, France)**

3

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17

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21

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27

28 **Abstract**

29 In port areas the identification of contamination sources is necessary for an efficient
30 management. Biomonitoring provides information on the environmental impact of the
31 pollutants. It is often difficult to differentiate the natural variations of biomarkers from those
32 induced by pollution. The present study aims to define a baseline level for biochemical
33 biomarkers in limpet (*Patella* sp.) collected in four North-Corsica port areas. Reference data
34 for five biomarkers (superoxide dismutase, glutathione S-transferase, laccase, pyruvate kinase
35 and acetylcholinesterase) were described in a model, using length of the limpet shell,
36 temperature and salinity. The measured biomarkers responses on potentially polluted sites
37 usually fell within the range of the expected values for an unaffected site, suggesting that a main
38 part of the variations is explained by environmental conditions. Not included in the model,
39 biological factors (sex, development stage, etc.), annual variation or other physico-chemical
40 parameter could explain the variations in the model.

41

42

43 Keywords: biomonitoring, baseline assessment criteria, biochemical biomarkers, *Patella* sp.

44

45

46 As a semi-enclosed sea affected by a growing coastal urbanization, the Mediterranean Sea is
47 the recipient of numerous anthropic pollutants (trace elements, organic and emergent
48 compounds). In addition to the pollutants coming from terrestrial discharges or anthropogenic
49 contamination (Benali et al., 2015; Lafabrie et al., 2008), port areas are also impacted by
50 recreational activities, adding further pressure on the ecosystem (Maisano et al., 2017). To set
51 up an effective environmental management of port areas, it is important to define the sources
52 of the pollution and its impacts on the ecosystem. This is why the monitoring of coastal
53 environment is essential for policy makers involved in the European Water Framework
54 Directive (WFD). However, monitoring natural marine ecosystems regarding chemical
55 mixtures is challenging and remains until today one of the major challenges in ecotoxicology.
56 Biomonitoring consists on the study of geographical and temporal trends of contaminants
57 through biological responses of an organism (Lacroix et al., 2015; Rainbow, 2002). It has been
58 developed to identify sources of pollution and prevent risks of future potential pollution
59 accidents. Compared to simple chemical water analysis, biomonitoring is a time-integrated
60 method and allows the evaluation of effects of contaminants on organism health (Lacroix et al.,
61 2015; Luna-Acosta et al., 2015; Rainbow, 2002; Wang, 2016). Limpets (*Patella* sp.) are more
62 and more used for biomonitoring studies and is considered as a promising bioindicator (Pérez
63 et al., 2019; Reguera et al., 2018) due the its abundance, its sedentary lifestyle, its large
64 distribution or its ease of sampling (Campanella et al., 2001; Pérez et al., 2019). Moreover, the
65 use of native biota as a monitoring tool for bioavailable pollutants in aquatic ecosystems was
66 demonstrated to constitute a highly valuable approach (Bertrand et al., 2018). Limpets are now
67 widely used in biomonitoring studies (Pérez et al., 2019; Reguera et al., 2018; Sánchez-Marín
68 et al., 2022; Viñas et al., 2018) and their high sensitivity to pollutants was demonstrated (Conti
69 et al., 2017; Reguera et al., 2018).

70 In environmental study, when a mixture of contaminants is present, it is recommended to use a
71 multi-biomarkers approach and to consider the environmental physicochemical parameters, to
72 differentiate the reversible effects from the irreversible ones (Garmendia et al., 2011). This
73 approach provides a better understanding and a more integrated view of the biological responses
74 in environmental risk assessment (Cravo et al., 2009; Ozkan et al., 2017). The efficiency of the
75 multi-biomarkers approach has been proven by several authors (Ait Alla et al., 2006; Damiens
76 et al., 2004; Garmendia et al., 2011; Guo et al., 2017; Vidal et al., 2002).

77 A wide diversity of biomarkers is used in a multi-biomarkers approach but the most common
78 are usually enzymes involved in antioxidative defences (Breitwieser et al., 2018; Ghribi et al.,
79 2020; Luna-Acosta et al., 2017b), in nervous system (Lionetto et al., 2013; Lionetto et al., 2003;
80 Matozzo et al., 2005), in immunity (Luna-Acosta et al., 2017a) or in energetic metabolism
81 (Falfushynska et al., 2019; Zebral et al., 2020). However, it is also demonstrated that biotic
82 factors (length, weight, reproductive cycle, etc.) (Louis et al., 2021), and abiotic factors
83 (temperature, salinity, etc.) (Castaldo et al., 2021; Prusina et al., 2014; Torossian et al., 2020;
84 Uluturhan et al., 2019; Zebral et al., 2020) can impact the biomarkers responses (Lomartire et
85 al., 2021). It is necessary to make a distinction between the fluctuations of biological responses
86 due to pollutants from the ones due to biotic or abiotic factors (Kalman et al., 2010).

87 Recently, several authors worked to define baseline data in biomarker using statistical analyses
88 with the standardization of data and the implementation of a predictive model (Barbarin et al.,
89 2022; Barrick et al., 2018; Baudou et al., 2021; Benito et al., 2019; Storhaug et al., 2019). The
90 Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR)
91 and ICES (The International Council for the Exploration of the Sea) developed a method
92 recommending the sampling of individuals at reference location over several seasons to record
93 the variations of confounding factors with the measure of biomarkers, and allowing the
94 identification of polluted locations (Amiard-Triquet et al., 2015; OSPAR, 2013). It then defines

95 the range of values expected in a reference site as the baseline level and values outside this
96 range could be classified into categories to define the range where environmental impacts are
97 expected. Several approaches were tested to define these baselines, and demonstrated the need
98 to use tools to identify anthropogenic and natural variations (Amiard-Triquet et al., 2015;
99 OSPAR, 2013). It is also important to characterize the relationship between confounding biotic
100 and abiotic factors to describe the influence of these parameters on the biomarker responses
101 (Barrick et al., 2016). An effective approach is based on a multiple polynomial regression model
102 and k-means clustering to standardize the influence of confounding factors on the biomarker
103 response, but the model needs to be chosen carefully (Barbarin et al., 2022; Barrick et al., 2018;
104 Burgeot et al., 2010).

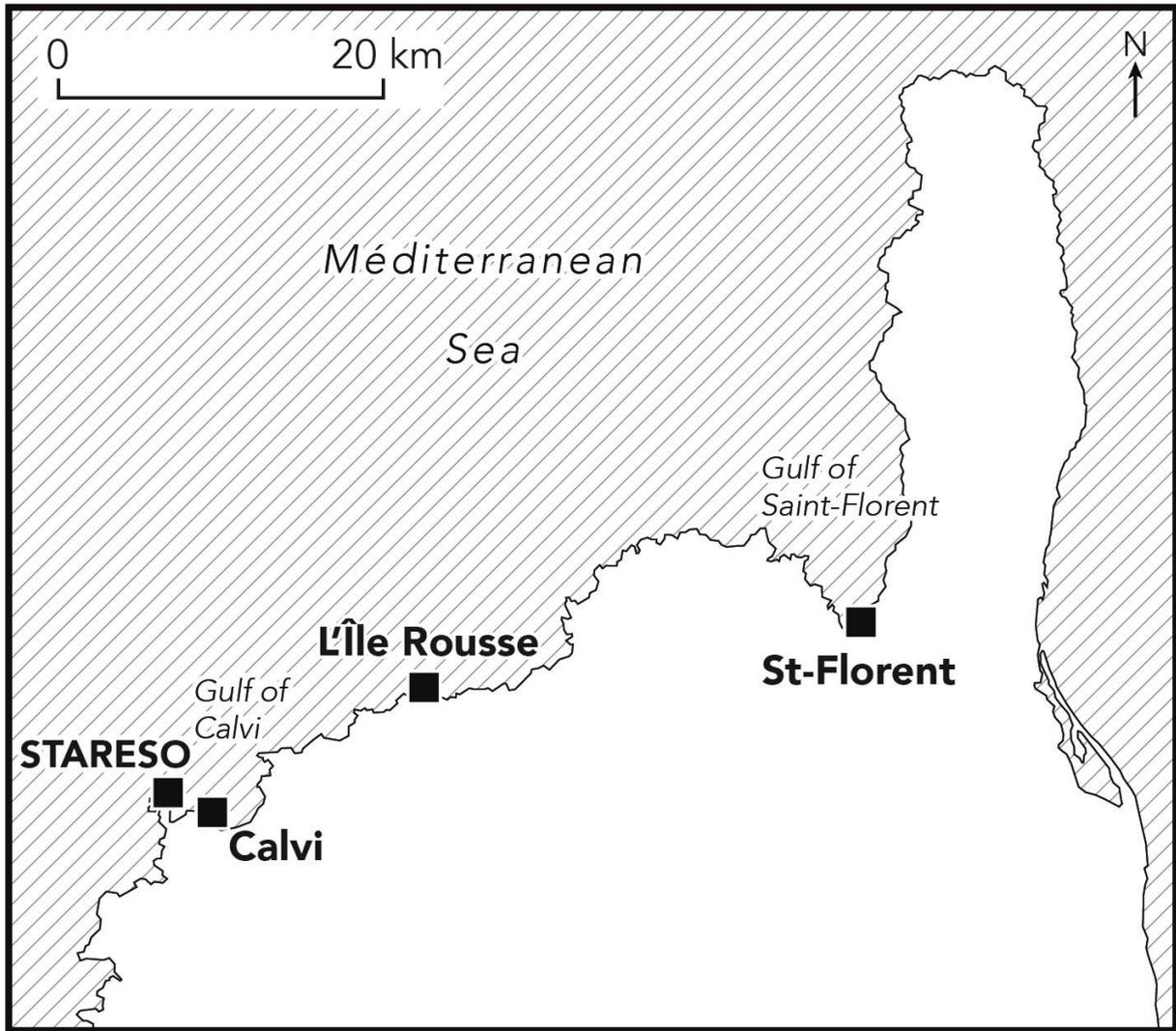
105

106 In the present study, the objective was to establish, through a predictive model, a baseline
107 assessment criterion for biochemical biomarkers in limpet used for port area biomonitoring in
108 North-Corsica. These additional data will provide more information on the ecological state of
109 the ecosystem in this area and will be used to improve the environmental management of the
110 ports.

111

112 St-Florent (N 42° 40' 50.362"; E 9° 17' 54.373"), Île Rousse (N 42° 38' 23.406"; E 8° 56' 7.778')
113 and Calvi (N 42° 33' 59.116"; E 8° 45' 29.094") ports were chosen as exposed locations
114 (Figure 1). The STARESO station in the Revellata bay (N 42° 34' 49.584"; E 8° 43' 31.511")
115 was chosen as the reference location (Figure 1) because it is distant from any city.

116



117

118

Figure 1 Map of the experimental locations in North Corsica (France).

119

120

At each sampling date, physicochemical parameter of water (temperature in °C and salinity in

121

PSU) were measured in the water column (~1 m of depth) using a CastAway CTD®

122

(Conductivity Temperature Depth) probe. Wild limpets ($n > 7$ per location) were sampled

123

during two years (September 2020, January 2021, September 2021 and January 2022).

124

Seasonal, temperature and salinity, as well as the shell length of limpets collected at each

125

sampling period are shown in Table 1.

126

127 Table 1: Shell length (mean \pm SD) of collected limpet, temperature and salinity observed at
 128 each sampling period for the different sampling locations

site	date	Sample			
		size	Shell length (cm)	Temperature (°C)	Salinity (PSU)
STARESO	September 2020	8	4,06 \pm 0,52	24,08	37,40
	January 2021	10	3,49 \pm 0,36	13,65	37,74
	September 2021	10	3,7 \pm 0,45	24,14	38,26
	January 2022	7	3,68 \pm 0,44	14,12	38,45
Calvi	September 2020	8	4,05 \pm 1,02	23,83	38,14
	January 2021	10	3,5 \pm 0,4	13,26	37,47
	September 2021	8	3,7 \pm 0,4	23,81	38,24
	January 2022	10	3,54 \pm 0,46	13,24	38,34
Île Rousse	September 2020	8	3,3 \pm 0,64	24,71	38,03
	January 2021	10	3,67 \pm 0,31	12,60	37,75
	September 2021	10	3,29 \pm 0,47	23,90	38,22
	January 2022	10	3,25 \pm 0,4	13,33	38,44
St Florent	September 2020	8	3,22 \pm 0,6	24,21	37,24
	January 2021	10	2,82 \pm 0,2	11,39	29,08
	September 2021	10	3,35 \pm 0,26	23,11	37,87
	January 2022	10	3,18 \pm 0,38	13,86	37,36

129

130

131 Individuals were collected on site ($n > 7$, see Table 1), immediately frozen in liquid nitrogen
 132 and kept at -80°C until further biochemical analyses. Back in the laboratory, the whole soft
 133 tissues of limpet were ground in liquid nitrogen using a MM400 Retsch[®] (GmbH, Éragny,
 134 Luxemburg) mixer mill and then hand homogenized on ice in one volume of phosphate buffered
 135 saline (PBS) solution (100 mM, pH 7.5) containing 0.1 % Triton X-100 and one mM
 136 ethylenediaminetetraacetic acid (EDTA) to avoid protein degradation. The samples were
 137 centrifuged 15 min at 12500 g at 4°C (Sorvall Legeng Micro 17R, ThermoFisher Scientific,
 138 Waltham, Massachusetts, USA). The supernatant was used for the subsequent biomarker
 139 analyses. The capacity to respond to oxidative stress was evaluated using superoxide dismutase
 140 (SOD - EC 1.15.1.1) activity using Paoletti et al. (1986)'s protocol. The activity of glutathione
 141 S-transferase (GST - EC 2.5.1.18), enzyme involved in detoxification process, was determined
 142 following Habig et al. (1974). The laccase (LAC - EC 1.10.3.2) is involved in the immune,

143 antioxidant and detoxification processes (Luna-Acosta et al., 2017a). The activity of this
144 enzyme was measured according to Luna-Acosta et al. (2010). The index of glycolysis
145 gluconeogenesis pathway were determined with the pyruvate kinase activity (PK - EC 2.7.1.40)
146 according to Childress and Somero (1979) as modified by Bailey et al. (2005).
147 Acetylcholinesterase (ACHE - EC 3.1.1.7) is widely used to estimate neurotoxic impacts of
148 pollutants, and its activity was assayed using Ellman et al. (1961)'s protocol. The total protein
149 content was measured with the bicinchoninic acid protein assay kit (Sigma-Aldrich®) that is
150 similar to the Lowry et al. (1951) procedure. Specific enzymatic activities were measured and
151 expressed as $U(\mu\text{mol}\cdot\text{min}^{-1})\cdot\text{mg}^{-1}$ of protein. All activities measurements were done on a
152 SpectroStar Nano spectrophotometer (BMG labtech, Champigny-sur-Marne, France) in
153 technical duplicate for each sample, using standard methods adapted for a microplate reader.
154 All chemicals were obtained from Sigma-Aldrich®.

155 Statistical analyses were performed using R software version 4.2.0. Based on the analysed
156 biomarkers (SOD, GST, LAC, PK and ACHE), data were organized by periods (4) and sites
157 (4). Normality and homogeneity of variances were respectively verified by Shapiro and Levene
158 tests. A series of observations were simulated according to mean values and standard deviation
159 of each biomarker. Confounding factors (two physicochemical water parameters: temperature
160 and salinity; and one biometric factor: shell length) and biomarker values of limpet collected in
161 the reference location (STARESO) were used to determine formula describing the relationship
162 between each biomarker and confounding factors (Press et al., 2007; Venables and Ripley,
163 2013). The chosen cofactors were considered as the most relevant for the present study and are
164 also often used in previous studies. Temperature and salinity were selected to consider the
165 influence of environmental conditions (Almeida et al., 2022; Benito et al., 2019) and the shell
166 length to consider the life stage of limpet.

167 Following this, pairwise analyses were conducted to identify which equation best described the
168 relationship between each biomarker and the confounding factors. This established a
169 polynomial regression formula. To address collinearity in the model, orthogonal polynomial
170 regression was used to obtain new regression models with uncorrelated variables and achieved
171 a final, stable polynomial regression equation for each biomarker:

172 **SOD (U.mg of protein⁻¹)** = 868.59 + 36.88 (length) - 0.98 (length)² + 0.01(length)³ - 166.3
173 (temperature) + 4.36 (temperature)² + 4.17 (salinity)

174 $R^2 = 0.62$

175 **GST (mU.mg of protein⁻¹)** = - 831.56 + 636.66 (length) - 16.79 (length)² + 0.15 (length)³ -
176 934.04 (temperature) + 24.1 (temperature)² + 42.1 (salinity)

177 $R^2 = 0.53$

178 **LAC (mU.mg of protein⁻¹)** = 1.8 + 0.01 (length) - 0.02 (temperature) - 0.03 (salinity)

179 $R^2 = 0.31$

180 **PK (mU.mg of protein⁻¹)** = 683.44 + 0.42 (length) - 0.77 (temperature) - 16.93 (salinity)

181 $R^2 = 0.21$

182 **ACHE (mU.mg of protein⁻¹)** = 75.71 - 0.03 (length) - 0.34 (temperature) - 1.61 (salinity)

183 $R^2 = 0.24$

184

185 With these equations, the predicted values were calculated for the reference location. The
186 residuals were obtained by subtracting the predicted values from the values obtained in the
187 laboratory. Using k-means clustering (Barrick et al., 2016; Barrick et al., 2018), the residuals
188 were classified into three groups that were used to determine the range of variations according
189 to environmental conditions and to define the baseline level (Table 2).

190

191 Table 2: Level of traffic light scheme (red: lethal effects expected, orange: sublethal effects
192 expected, yellow: range expected on non-impacted sites, green: evidence of good health) used

193 to define the range of environmental effects. Y indicates the mean value for the reference site
 194 (STARESO). SOD (superoxide dismutase), GST (gluthathione-S-transferase), LAC (laccase
 195 type phenoloxidase), PK (pyruvate kinase), ACHE (acetylcholinesterase).

Indicators	SOD residuals	GST residuals	LAC residuals	PK residuals	ACHE residuals
Green	< y - 33.21 [y - 33.21; y +	< y - 149.61 [y - 149.61; y +	< y - 0.30 [y - 0.30; y +	< y - 20.85 [y - 20.85; y +	< y - 3.69 [y - 3.69; y +
Yellow	83.68] [y + 83.68; y +	320.25] [y + 320.25; y +	0.20] [y + 0.20; y +	13.21] [y + 13.21; y +	2.36] [y + 2.36; y +
Orange	90.43]	344.56]	0.54]	169.99]	20.12]
Red	> y + 90.43	> y + 344.56	> y + 0.54	> y + 169.99	> y + 20.12

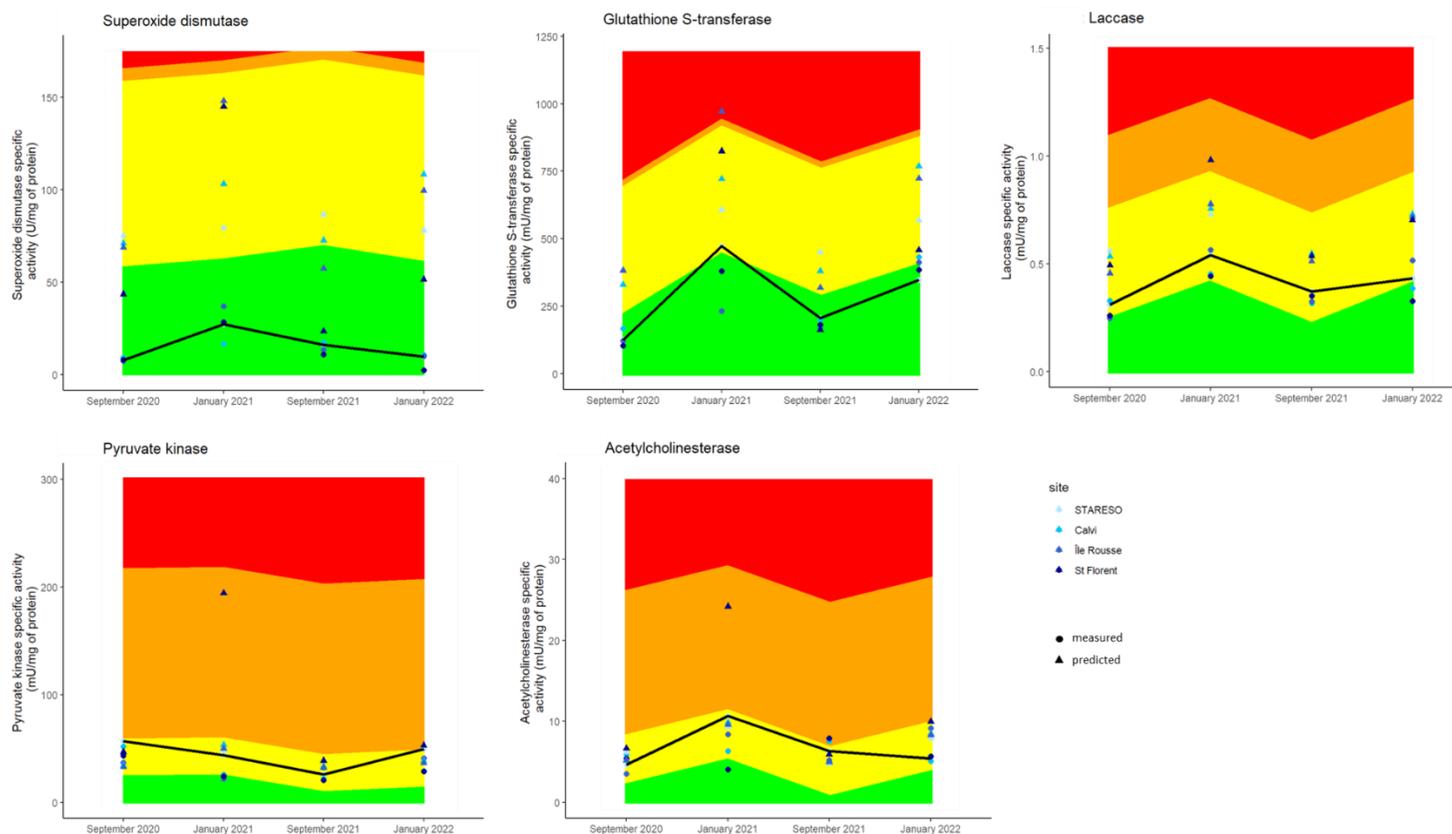
196
 197 The equations were then used to calculate the predicted value for each biomarker on each
 198 location. These values and the average biomarker activity for the reference location (black line)
 199 were graphically represented within the range of variation for each biomarker (Figure 2). The
 200 interpretation of the data should be taken with precaution, because statistical analyses
 201 considering maximum of variable is always complex to explain and error of interpretation can
 202 occur. However, for environmental studies, it is essential to consider the influence of
 203 environmental parameters with time and location. It is also essential to take into account
 204 morphometric parameter of the individual as they are often collected as wild in the environment.

205
 206 The model displayed the seasonal variations of the biomarker baseline. In general, the activity
 207 of the biomarkers is lower in winter. This winter decrease is consistent with previous studies
 208 results (Barbarin et al., 2022; Barrick et al., 2018; Storhaug et al., 2019) and could be explain
 209 by several factors. The lower temperatures in winter could induce a slower metabolism and
 210 subsequently lower enzymes activities. Seasonal variations in could also be related to the
 211 reproductive state of organisms as it was observed for mussels by Storhaug et al. (2019). Having
 212 more information on the seasonal variations of biomarkers could help to improve biomonitoring
 213 of specific environments such as ports areas. In the ports selected in the present study, the
 214 decrease observed in winter should be considered. It would be necessary to obtain a more

215 precise view of the variation along the year by collecting additional samples during the other
216 period of the year and to do so for a longer period of time. After that, biomonitoring of the port
217 area should be realized during months that are comparable to each other (with the same
218 baseline) to observe the impact of pollution and not the natural variation of biomarkers.

219

220 The results showed that the observed values for all the biomarkers are very close to the value
221 observed for the reference site (STARESO) and fell within the green or yellow ranges, showing
222 either evidence of good health or unimpacted organisms (Figure 2). For PK and ACHE, the
223 values predicted by the model were also really close to the observed values. This reflects good
224 predictions of the model for these two biomarkers. The only exceptions for PK and ACHE are
225 the values predicted at St-Florent in January 2021. This value is much higher than the other
226 ones and fell between the orange range where sublethal effects are expected. For the SOD, GST
227 and LAC, the observed values also fell pretty close to the value observed for the reference site,
228 so it is still a good sign for the ecological status of the organisms in North Corsica ports.
229 However, the difference between observed and predicted values are more important for these
230 three biomarkers. Despite this difference, the observed value fell most of the time within the
231 yellow range where no impacts should be detected on organism. In January 2021, the predicted
232 values for LAC in St-Florent and GST in Île Rousse are the exceptions, falling respectively in
233 the orange (sublethal effects expected) and red range (lethal effects expected). The difference
234 between observed and predicted values for SOD, GST and LAC is slightly more important in
235 winter, especially in January 2021.



236

237 **Figure 2** Graphical representation of health indicator (red: lethal effects expected, orange: sublethal effects expected, yellow: range expected on
 238 non-impacted sites, green: evidence of good health) for superoxide dismutase, glutathione-S-transferase, laccase type phenoloxidase, pyruvate
 239 kinase and acetylcholinesterase in STARESO (reference location), Calvi, Île Rousse and St Florent. For each location, the observed and predicted
 240 (from the equations defined in the present study) values are respectively represented by circles and triangles. The black line represents the
 241 observed value for the reference location (STARESO).

242 The statistical model works pretty well. For most of the data and especially for PK and ACHE,
243 the predicted values are consistent with the observed ones. These results confirm that the
244 equations of the model were correctly selected. However, the model still needs some
245 improvements for the SOD, LAC and GST, and this is especially true for the winter periods
246 (2021 and 2022) and the St-Florent port. The different results between the observed values and
247 the model predictions could be explained by several possibilities. The choice of cofounding
248 factors incorporated into the model can influence the results. The environmental factors
249 included in the present model are the most common in similar studies (Barbarin et al., 2022;
250 Barrick et al., 2016; Barrick et al., 2018) but can also change to a more or less extent from one
251 year to another. However, further investigations are necessary to identify what else can
252 influence the biomarkers values (nutrient availability, reproductive status, life stage, light,
253 presence of contaminants, etc.) (Barrick et al., 2016; Barrick et al., 2018). For example, St-
254 Florent port is characterized by the presence of the Aliso River mouth. A higher input of
255 freshwater in this port probably has an influence on the biomarker responses and should be
256 taken into consideration in a future model.

257 Additional factors could be easily incorporated into the model but it will be necessary to
258 evaluate the covariation between these factors. This can be rather difficult as some of these can
259 be strongly linked (e.g. nutrient availability and light, life stage and weight, etc.) (Barrick et al.,
260 2018). Once the characterization of the cofactor's correlation included into the model, it can be
261 used discrimination between cofounding factors and to identify the impacts of contaminants.
262 For futures studies and to improve the precision of the model, it will be important to collect
263 more organisms to increase the number of samples and to run the analysis for a longer time
264 period to consider the variations between years.

265

266 The objective of this study was to design a baseline level for biomarkers that account for
267 confounding factors. By providing more data on the ecological status of port areas, the
268 development of this model for limpets will help the implementation of biomonitoring
269 environmental program in North Corsica ports. Already developed for ragworms (Barrick et
270 al., 2016; Barrick et al., 2018), bivalve (Barbarin et al., 2022; Storhaug et al., 2019) or fish
271 (Baudou et al., 2021), this kind of statistical approach provide a better understanding of the
272 biomarkers responses. The present study is the first to develop this model on limpet. It also
273 brings more data for the use of this specie in biomonitoring program, as it was shown to be a
274 promising bioindicator in previous studies (Mbandzi et al., 2022; Pérez et al., 2019; Sánchez-
275 Marín et al., 2022).

276

277

- 279 Ait Alla, A., Mouneyrac, C., Durou, C., Moukrim, A., Pellerin, J., 2006. Tolerance and
280 biomarkers as useful tools for assessing environmental quality in the Oued Souss estuary
281 (Bay of Agadir, Morocco). *Comparative Biochemistry and Physiology Part C: Toxicology*
282 *and Pharmacology* 143, 23-29.
- 283 Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Soares, A.M.V.M., Freitas, R., 2022.
284 Salinity-dependent impacts on the effects of antiepileptic and antihistaminic drugs in
285 *Ruditapes philippinarum*. *Sci. Total Environ.* 806, 150369.
- 286 Amiard-Triquet, C., Amiard, J.C., Mouneyrac, C., 2015. Aquatic ecotoxicology: advancing
287 tools for dealing with emerging risks. Academic press.
- 288 Bailey, D.M., Genard, B., Collins, M.A., Rees, J.-F., Unsworth, S.K., Battle, E.J.V., Bagley,
289 P.M., Jamieson, A.J., Priede, I.G., 2005. High swimming and metabolic activity in the deep-
290 sea eel *Synaphobranchus kaupii* revealed by integrated in situ and in vitro measurements.
291 *Physiol. Biochem. Zool.* 78, 335-346.
- 292 Barbarin, M., Muttin, F., Thomas, H., 2022. First study on the determination of baseline
293 biomarkers in *Mimachlamys varia* for an intra-port environmental biomonitoring in French
294 Atlantic coastline (La Rochelle). *Mar. Pollut. Bull.* 182, 113979.
- 295 Barrick, A., Châtel, A., Marion, J.-M., Perrein, H., Bruneau, M., Mouneyrac, C., 2016. A novel
296 methodology for the determination of biomarker baseline levels in the marine polychaete
297 *Hediste diversicolor*. *Mar. Pollut. Bull.* 108.
- 298 Barrick, A., Marion, J.-M., Perrein-Ettajani, H., Châtel, A., Mouneyrac, C., 2018. Baseline
299 levels of biochemical biomarkers in the endobenthic ragworm *Hediste diversicolor* as useful
300 tools in biological monitoring of estuaries under anthropogenic pressure. *Mar. Pollut. Bull.*
301 129, 81-85.
- 302 Baudou, F.G., Eissa, B.L., Ossana, N.A., Mastrángelo, M.M., Ferro, J.P., Campos, L.B., Ferrari,
303 L., 2021. First baseline for bioenergetic biomarkers in *Cnesterodon decemmaculatus* as test
304 organism in ecotoxicological studies. *Ecotoxicol. Environ. Saf.* 208, 111639.
- 305 Benali, I., Boutiba, Z., Merabet, A., Chèvre, N., 2015. Integrated use of biomarkers and
306 condition indices in mussels (*Mytilus galloprovincialis*) for monitoring pollution and
307 development of biomarker index to assess the potential toxic of coastal sites. *Mar. Pollut.*
308 *Bull.* 95, 385-394.
- 309 Benito, D., Ahvo, A., Nuutinen, J., Bilbao, D., Saenz, J., Etxebarria, N., Lekube, X., Izagirre,
310 U., Lehtonen, K.K., Marigómez, I., Zaldibar, B., Soto, M., 2019. Influence of season-
311 depending ecological variables on biomarker baseline levels in mussels (*Mytilus trossulus*)
312 from two Baltic Sea subregions. *Sci. Total Environ.* 689, 1087-1103.
- 313 Bertrand, L., Monferrán, M.V., Mouneyrac, C., Amé, M.V., 2018. Native crustacean species as
314 a bioindicator of freshwater ecosystem pollution: A multivariate and integrative study of
315 multi-biomarker response in active river monitoring. *Chemosphere* 206, 265-277.
- 316 Breitwieser, M., Dubillot, E., Barbarin, M., Churlaud, C., Huet, V., Muttin, F., Thomas, H.,
317 2018. Assessment of the biological quality of port areas: A case study on the three harbours
318 of La Rochelle: The marina, the fishing harbour and the seaport. *PLOS ONE* 13, e0198255.
- 319 Burgeot, T., Gagné, F., Forget-Ieray, J., Bocquené, G., 2010. Acetylcholinesterase:
320 methodology development of a biomarker and challenges of its application for
321 biomonitoring. *Aquat. Living Resour.* 17, 309-316.
- 322 Campanella, L., Conti, M.E., Cubadda, F., Supapane, C., 2001. Trace metals in seagrass, algae
323 and molluscs from an uncontaminated area in the Mediterranean. *Environ. Pollut.* 111, 117-
324 126.

325 Castaldo, G., Pillet, M., Ameryckx, L., Bervoets, L., Town, R.M., Blust, R., De Boeck, G.,
326 2021. Temperature effects during a sublethal chronic metal mixture exposure on common
327 carp (*Cyprinus carpio*). *Frontiers in Physiology* 12.

328 Childress, J.J., Somero, G.N., 1979. Depth-related enzymic activities in muscle, brain and heart
329 of deep-living pelagic marine teleosts. *Mar. Biol.* 52, 273-283.

330 Conti, M.E., Mele, G., Finioia, M.G., 2017. Baseline trace metals in *Patella caerulea* in a central
331 Tyrrhenian ecosystem (Pontine Islands archipelago and Lazio region coastal sites, Italy).
332 *Environmental Science and Pollution Research* 24, 8852-8865.

333 Cravo, A., Lopes, B., Serafim, Â., Company, R., Barreira, L., Gomes, T., Bebianno, M.J., 2009.
334 A multibiomarker approach in *Mytilus galloprovincialis* to assess environmental quality. *J.*
335 *Environ. Monit.* 11, 1673-1686.

336 Damiens, G., His, E., Gnassia-Barelli, M., Quiniou, F., Roméo, M., 2004. Evaluation of
337 biomarkers in oyster larvae in natural and polluted conditions. *Comparative Biochemistry*
338 *and Physiology Part C: Toxicology and Pharmacology* 138, 121-128.

339 Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid
340 colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7, 88-95.

341 Falfushynska, H., Sokolov, E.P., Haider, F., Oppermann, C., Kragl, U., Ruth, W., Stock, M.,
342 Glufke, S., Winkel, E.J., Sokolova, I.M., 2019. Effects of a common pharmaceutical,
343 atorvastatin, on energy metabolism and detoxification mechanisms of a marine bivalve
344 *Mytilus edulis*. *Aquat. Toxicol.* 208, 47-61.

345 Garmendia, L., Soto, M., Ortiz-Zarragoitia, M., Orbea, A., Cajaraville, M.P., Marigómez, I.,
346 2011. Application of a battery of biomarkers in mussel digestive gland to assess long-term
347 effects of the Prestige oil spill in Galicia and Bay of Biscay: Correlation and multivariate
348 analysis. *J. Environ. Monit.* 13, 933-942.

349 Ghribi, F., Richir, J., Bejaoui, S., Boussoufa, D., Marengo, M., El Cafsi, M.h., Gobert, S., 2020.
350 Trace elements and oxidative stress in the Ark shell *Arca noae* from a Mediterranean coastal
351 lagoon (Bizerte lagoon, Tunisia): are there health risks associated with their consumption?
352 *Environmental Science and Pollution Research*.

353 Guo, R., Pan, L., Ji, R., 2017. A multi-biomarker approach in scallop *Chlamys farreri* to assess
354 the impact of contaminants in Qingdao coastal area of China. *Ecotoxicol. Environ. Saf.* 142,
355 399-409.

356 Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic
357 step in mercapturic acid formation. *J. Biol. Chem.* 249, 7130-7139.

358 Kalman, J., Buffet, P.E., Amiard, J.C., Denis, F., Mouneyrac, C., Amiard-Triquet, C., 2010.
359 Assessment of the influence of confounding factors (weight, salinity) on the response of
360 biomarkers in the estuarine polychaete *Nereis diversicolor*. *Biomarkers* 15, 461-469.

361 Lacroix, C., Richard, G., Segueineau, C., Guyomarch, J., Moraga, D., Auffret, M., 2015. Active
362 and passive biomonitoring suggest metabolic adaptation in blue mussels (*Mytilus* spp.)
363 chronically exposed to a moderate contamination in Brest harbor (France). *Aquat. Toxicol.*
364 162, 126-137.

365 Lafabrie, C., Pergent-Martini, C., Pergent, G., 2008. Metal contamination of *Posidonia*
366 *oceanica* meadows along the Corsican coastline (Mediterranean). *Environ. Pollut.* 151, 262-
367 268.

368 Lionetto, M.G., Caricato, R., Calisi, A., Giordano, M.E., Schettino, T., 2013.
369 Acetylcholinesterase as a biomarker in environmental and occupational medicine: new
370 insights and future perspectives. *BioMed Research International* 2013, 321213.

371 Lionetto, M.G., Caricato, R., Giordano, M.E., Pascariello, M.F., Marinosci, L., Schettino, T.,
372 2003. Integrated use of biomarkers (acetylcholinesterase and antioxidant enzymes activities)
373 in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. *Mar.*
374 *Pollut. Bull.* 46, 324-330.

375 Lomartire, S., Marques, J.C., Gonçalves, A.M.M., 2021. Biomarkers based tools to assess
376 environmental and chemical stressors in aquatic systems. *Ecol. Indicators* 122, 107207.

377 Louis, F., Delahaut, L., Gaillet, V., Bonnard, I., Paris-Palacios, S., David, E., 2021. Effect of
378 reproduction cycle stage on energy metabolism responses in a sentinel species (*Dreissena*
379 *polymorpha*) exposed to cadmium: What consequences for biomonitoring? *Aquat. Toxicol.*
380 230, 105699.

381 Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the
382 Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.

383 Luna-Acosta, A., Breitwieser, M., Renault, T., Thomas-Guyon, H., 2017a. Recent findings on
384 phenoloxidases in bivalves. *Mar. Pollut. Bull.* 122, 5-16.

385 Luna-Acosta, A., Bustamante, P., Budzinski, H., Huet, V., Thomas-Guyon, H., 2015. Persistent
386 organic pollutants in a marine bivalve on the Marennes-Oléron Bay and the Gironde Estuary
387 (French Atlantic Coast) - Part 2: Potential biological effects. *Sci. Total Environ.* 514, 511-
388 522.

389 Luna-Acosta, A., Bustamante, P., Thomas-Guyon, H., Zaldibar, B., Izagirre, U., Marigómez,
390 I., 2017b. Integrative biomarker assessment of the effects of chemically and mechanically
391 dispersed crude oil in Pacific oysters, *Crassostrea gigas*. *Sci. Total Environ.* 598, 713-721.

392 Luna-Acosta, A., Rosenfeld, E., Amari, M., Fruitier-Arnaudin, I., Bustamante, P., Thomas-
393 Guyon, H., 2010. First evidence of laccase activity in the Pacific oyster *Crassostrea gigas*.
394 *Fish Shellfish Immunol.* 28, 719-726.

395 Maisano, M., Cappello, T., Natalotto, A., Vitale, V., Parrino, V., Giannetto, A., Oliva, S.,
396 Mancini, G., Cappello, S., Mauceri, A., Fasulo, S., 2017. Effects of petrochemical
397 contamination on caged marine mussels using a multi-biomarker approach: Histological
398 changes, neurotoxicity and hypoxic stress. *Mar. Environ. Res.* 128, 114-123.

399 Matozzo, V., Tomei, A., Marin, M.G., 2005. Acetylcholinesterase as a biomarker of exposure
400 to neurotoxic compounds in the clam *Tapes philippinarum* from the Lagoon of Venice. *Mar.*
401 *Pollut. Bull.* 50, 1686-1693.

402 Mbandzi, N., Vincent Nakin, M.D., Oyedeji, A.O., 2022. Temporal and spatial variation of
403 heavy metal concentration in four limpet species along the southeast coast of South Africa.
404 *Environ. Pollut.* 302, 119056.

405 OSPAR, 2013. Background document and technical annexes for biological effects monitoring,
406 The Convention for the Protection of the Marine Environment of the North-East Atlantic.

407 Ozkan, D., Dagdeviren, M., Katalay, S., Guner, A., Yavaşoğlu, N.Ü.K., 2017. Multi-biomarker
408 responses after exposure to pollution in the Mediterranean mussels (*Mytilus*
409 *galloprovincialis* L.) in the Aegean coast of Turkey. *Bull. Environ. Contam. Toxicol.* 98, 46-
410 52.

411 Paoletti, F., Aldinucci, D., Mocali, A., Caparrini, A., 1986. A sensitive spectrophotometric
412 method for the determination of superoxide dismutase activity in tissue extracts. *Anal.*
413 *Biochem.* 154, 536-541.

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

417 Press, W.H., Teukolsky, S.A., Vetterling, W.T., Flannery, B.P., 2007. Numerical recipes 3rd
418 edition: The art of scientific computing. Cambridge university press.

419 Prusina, I., Sarà, G., De Pirro, M., Dong, Y.-W., Han, G.-D., Glamuzina, B., Williams, G.A.,
420 2014. Variations in physiological responses to thermal stress in congeneric limpets in the
421 Mediterranean Sea. *J. Exp. Mar. Biol. Ecol.* 456, 34-40.

422 Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what?
423 *Environ. Pollut.* 120, 497-507.

424 Reguera, P., Couceiro, L., Fernández, N., 2018. A review of the empirical literature on the use
425 of limpets *Patella* spp. (Mollusca: Gastropoda) as bioindicators of environmental quality.
426 Ecotoxicol. Environ. Saf. 148, 593-600.

427 Sánchez-Marín, P., Schultze, F., Besada, V., 2022. Use of limpets as alternative to mussels in
428 metal pollution monitoring; application in the Canary Islands. Environ. Pollut. 308, 119614.

429 Storhaug, E., Nahrgang, J., Pedersen, K.B., Brooks, S.J., Petes, L., Bakhmet, I.N., Frantzen,
430 M., 2019. Seasonal and spatial variations in biomarker baseline levels within Arctic
431 populations of mussels (*Mytilus* spp.). Sci. Total Environ. 656, 921-936.

432 Torossian, J.L., Hosek, K.E., Donelan, S.C., Trussell, G.C., Helmuth, B.S., Zippay, M.L., 2020.
433 Physiological and biochemical responses to acute environmental stress and predation risk in
434 the blue mussel, *Mytilus edulis*. J. Sea Res. 159, 101891.

435 Uluturhan, E., Darılmaz, E., Kontas, A., Bilgin, M., Alyuruk, H., Altay, O., Sevgi, S., 2019.
436 Seasonal variations of multi-biomarker responses to metals and pesticides pollution in *M.*
437 *galloprovincialis* and *T. decussatus* from Homa Lagoon, Eastern Aegean Sea. Mar. Pollut.
438 Bull. 141, 176-186.

439 Venables, W.N., Ripley, B.D., 2013. Modern applied statistics with S-PLUS. Springer Science
440 & Business Media.

441 Vidal, M.-L., Bassères, A., Narbonne, J.-F., 2002. Seasonal variations of pollution biomarkers
442 in two populations of *Corbicula fluminea* (Müller). Comparative Biochemistry and
443 Physiology Part C: Toxicology & Pharmacology 131, 133-151.

444 Viñas, L., Pérez-Fernández, B., Soriano, J.A., López, M., Bargiela, J., Alves, I., 2018. Limpet
445 (*Patella* sp) as a biomonitor for organic pollutants. A proxy for mussel? Mar. Pollut. Bull.
446 133, 271-280.

447 Wang, W.X., 2016. Chapter 4 - Bioaccumulation and Biomonitoring, in: Blasco, J., Chapman,
448 P.M., Campana, O., Hampel, M. (Eds.), Marine Ecotoxicology. Academic Press, pp. 99-119.

449 Zebral, Y.D., da Silva Fonseca, J., Roza, M., Costa, P.G., Robaldo, R.B., Bianchini, A., 2020.
450 Combining elevated temperature with waterborne copper: Impacts on the energy metabolism
451 of the killifish *Poecilia vivipara*. Chemosphere 253, 126631.

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455 **Statements and declarations**

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

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466 **Authors Contributions**

467 Marion Pillet: conceptualization, methodology, software, validation, data collection, formal
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469 Frédéric Muttin: conceptualization, methodology, software, validation, formal analysis,
470 investigation, visualization.

471 Michel Marengo: data collection, project administration, funding acquisition

472 Lovina Fullgrabe: data collection, formal analysis.

473 Valérie Huet: formal analyses.

474 Pierre Lejeune: conceptualization, funding acquisition.

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

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477 **Ethical Approval**

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

479

480 **Consent to Participate**

481 Yes

482

483 **Consent to Publish**

484 Yes

485

486 **Availability of data and materials**

487 The datasets generated during and/or analysed during the current study are available from the
488 corresponding author on reasonable request.

489

490 List of captions:

491 **Fig. 1** Map of the experimental locations in North Corsica (France)

492

493 **Fig. 2** Graphical representation of health indicator (red: lethal effects expected, orange:
494 sublethal effects expected, yellow: range expected on non-impacted sites, green: evidence of
495 good health) for superoxide dismutase, glutathione-S-transferase, laccase type phenoloxidase,
496 pyruvate kinase and acetylcholinesterase in STARESO (reference location), Calvi, Île Rousse
497 and St Florent. For each location, the observed and predicted (from the equations defined in the
498 present study) values are respectively represented by circles and triangles. The black line
499 represents the observed value for the reference location (STARESO).

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