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1 **Seasonal change in trace element concentrations of *Paracentrotus lividus*: its**
2 **use as a bioindicator**

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15

16 **Highlights**

- 17 - Importance physiological factors have been identified
18
19 - Gonadosomatic index is the variable most closely related to temporal variations
20
21 - Trace elements contamination is lower during the summer season
22
23 - The use of gonads should be avoided during the spawning period (autumn/winter)

24 **Abstract**

25 An assessment of classical and emerging trace element contamination was conducted on gonads of
26 the sea urchin *Paracentrotus lividus* (Lamarck, 1819), in Corsica (Western Mediterranean). The
27 aim of this study was to evaluate the contamination levels at different sites by following the
28 seasonal variation of 22 trace elements. The sea urchins analyzed were taken in 2017 from reference
29 and more impacted sites in four Corsican areas. The results obtained shown the importance of biotic
30 factors such as gender, reproduction and the way of life. Variations have been highlighted with
31 lower trace element concentrations during the summer season. This is mainly due to a dilution
32 phenomenon resulting from gametogenesis. The pollution index (TEPI) was determined and
33 highlighted differences in contamination levels at the various sites. This work could provide
34 additional support for other tools for the diagnosis and monitoring of coastal water quality. It
35 provides useful new data to enable managers to act at the source and reduce degradation in order
36 to improve the ecological quality of marine waters.

37 **Keywords:** Trace elements, *Paracentrotus lividus*, Bioindicator, Seasonal variation

38 **1. Introduction**

39 The growth of industrial, agricultural and urban activities gives rise to the introduction of
40 considerable amounts of chemicals in marine coastal ecosystems. These substances have toxic
41 properties likely to cause extensive damage at the scale of organisms, populations and ecosystems
42 (Nordberg *et al.*, 2007; Amiard, 2011). Furthermore, intensive human activities, particularly in
43 coastal areas, have a major environmental impact on these productive zones (Papathanassiou and
44 Gabrielides, 1999). The United Nations Environment Programme estimated that 650 million tons
45 of sewage, 129,000 tons of mineral oil, 60,000 tons of mercury, 3,800 tons of lead and 36,000 tons
46 of phosphates are dumped into the Mediterranean each year. In addition, 70 per cent of the
47 wastewater dumped into the Mediterranean is untreated. These pressures make the Mediterranean
48 a vulnerable ecological unit (Turley, 1999). Furthermore, as the water bodies renews with a few
49 decades in the Mediterranean versus a few centuries for the ocean, this sea is a veritable laboratory
50 for observing the pressures and changes that humans exert on the environment (Bethoux *et al.*,
51 1999).

52 Trace elements (TEs) are among the most common contaminants in the marine ecosystem. Due to
53 their toxicity, persistence and ability to accumulate in marine organisms, they are considered as
54 serious pollutants in marine ecosystems (Bonanno and Di Martino, 2017). TEs are present in the
55 different compartments of the environment at low concentrations (Baize, 2009). In the marine
56 environment, they can remain in solution, be adsorbed on sedimentary particles, precipitate to the
57 bottom, or be bioaccumulated or biomagnified by organisms and to reach concentrations that can
58 be toxic (Warnau *et al.*, 1998). Above a certain threshold, all TEs present a potential danger that
59 can cause disturbances at cellular level, individual level, and also population or ecosystem levels
60 (Amiard, 2011). They represent a potential danger for marine organisms (*e.g.* Allemand *et al.*,
61 1989; Walter *et al.*, 1989) and for human consumers of sea urchin gonads. As a result of the threats

62 posed by TEs in the environment, they must be continuously monitored from their emission sources
63 to their final deposition in the oceans (Richir and Gobert, 2014).

64 In order to assess the levels of contaminants available in the ecosystem, organisms can be used as
65 bioindicators. Postmetamorphic echinoids in general, and *Paracentrotus lividus* (Lamarck, 1816),
66 in particular, are interesting candidates for the bioindication of TEs contaminations and have
67 already been used in the Mediterranean and elsewhere (*e.g.* Augier *et al.*, 1989; Guendouzi *et al.*,
68 2017; Ternengo *et al.*, 2018). Due to its wide distribution, abundance in several coastal ecosystems,
69 ease of harvesting, longevity, relative sedentarity and high tolerance of pollutants, *Paracentrotus*
70 *lividus* is an organism that is recognized for its role as bioindicator (Warnau *et al.*, 1998; Geraci *et*
71 *al.*, 2004. Salvo *et al.*, 2014). Its gonads and digestive tract are described as the organs accumulating
72 the most trace elements (Augier *et al.*, 1989), thus, the study of their ecotoxicological properties is
73 also of public health interest (Salvo *et al.*, 2015). Trace elements concentration in the gonads is
74 known to vary according to different biological (age, gender), physiological (reproduction) or
75 environmental (season) factors (Warnau *et al.*, 1998; Guendouzi *et al.*, 2017; Rocha *et al.*, 2019).
76 The elementary constitution of the sea urchin reflects the composition of its environment and
77 provides a basis for monitoring the patterns of change of contamination (Morrison *et al.*, 2017).

78 In the Mediterranean, numerous studies using *Paracentrotus lividus* as a bioindicator have studied
79 classical TEs such as zinc or lead, but there has been little or no description of the contamination
80 related to emerging trace elements (*e.g.* Rouane-Hacene *et al.*, 2017; Guendouzi *et al.*, 2017). Well-
81 known throughout the Mediterranean region, *Paracentrotus lividus* is a species of economic and
82 ecological importance (Lawrence and Sammarco, 1982; Kelly, 2004). The sea urchin is of high
83 commercial value and represents a complementary resource for artisanal fishing. The taste quality
84 of its gonads also makes it a species appreciated and targeted by recreational fishing. In addition,
85 as a primary consumer, it plays a key role in the structuring and functioning of benthic ecosystems
86 and more particularly of macrophyte communities (Lawrence and Sammarco, 1982).

87 Corsica island is often considered as a 'pristine' region on account of its water quality and the low
88 anthropic pressure (Lafabrie *et al.*, 2008; Gobert *et al.*, 2017; Marengo *et al.*, 2018). Nevertheless,
89 according to recent studies, local contamination similar to that recorded in other anthropized areas
90 in the Mediterranean can be found (Richir *et al.*, 2015), with areas classified on the basis of different
91 levels of contamination as anthropized or preserved sites (Ternengo *et al.*, 2018). This is a real
92 asset that makes it a particularly suitable study area to identify contaminants and to monitor their
93 dynamics according to anthropogenic pressures.

94 The purpose of this study is (i) to monitor the spatio-temporal dynamics of 22 TEs (classical and
95 emerging) in sea urchin gonads collected along the Corsican coasts, and (ii) to evaluate the seasonal
96 patterns of change in the pollution index characterizing each site to determine whether these
97 variations are linked to the sea urchin's physiology or to contamination. This paper will assess the
98 bio-indicator potential of sea urchins and compare this model with other bioindicators.

99 **2. Material and Methods**

100 **2.1 Sampling sites, collection and preparation of samples**

101 Sea urchin samples were collected in May, August and November 2017, and in order to have a
102 complete range of seasonal monitoring, the February 2017 data of Ternengo *et al.* (2018) were
103 added. Sea urchins were collected in the Western Mediterranean Sea in four Corsican coastal areas
104 between 1 and 5 m depth (Figure 1). In each area, two sites were defined, diverging by their
105 ecological characteristics and their degree of anthropization: (1) a reference site, chosen for its
106 distance from any pollution source and supposed to have a good ecological status, and (2) a site
107 close to identified anthropogenic sources (wastewater treatment plant, commercial harbour, marina
108 and a former asbestos mine) supposedly impacted.

109 Thirty sea urchins were collected in each area, 15 per site for each season, resulting in a total of
110 480 individuals harvested in this study. After measuring height and weight, the sea urchins were
111 dissected and the gender was determined. The sex ratio has been respected, to the extent possible,
112 to avoid bias. The gonads were removed and weighed to calculate the gonadosomatic index of each
113 individual. This index was calculated using the following formula: $(GFW/TFW) * 100$ where *GFW*
114 is the gonad fresh weight and *TFW* is total fresh weight. Gonads were cleaned with ultrapure water
115 and stored at -20°C.

116 **2.2 Trace element analysis**

117 Prior to the analysis, samples were lyophilized (CHRIST LCG Lyochamber Guard 121550
118 PMMA/Alpha 1-4 LD plus) and ground in an agate mortar. Approximately 0.2 g of each dried
119 material was mineralized in a closed microwave digestion labstation (Ethos D Milestone Inc.),
120 using nitric acid and hydrogen peroxide as reagents (suprapur grade, Merck). The TE
121 concentrations were determined by Inductively Coupled Plasma Mass Spectrometry using
122 Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, Perkin Elmer), according to the

123 method described by Richir and Gobert (2014). A total of 22 trace elements were analyzed: silver
124 (Ag), aluminium (Al), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd),
125 cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), manganese (Mn), molybdenum
126 (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), uranium (U), vanadium (V),
127 and zinc (Zn). In order to check the purity of the chemicals used, a large number of chemical
128 blanks were run every 40 samples. Analytical quality control was achieved using Certified
129 Reference Materials (CRM), DORM-4 (fish protein), NIST 1566b (oyster) and NIST 2976 (mussel
130 tissue). For each TE, detection limit (LD) and quantification limit (LQ) were calculated according
131 to Currie (1999) and Grinzaid *et al.* (1977) depending on their specific blank distribution. The
132 results are expressed in milligrams of element per kilogram of dry weight \pm standard error (mg.kg⁻¹
133 dw \pm se). TEs with values generally below the detection limit were removed from the database.
134 For the others, concentrations below the LD were replaced with a value of LD/2, as reported by
135 Skrbić *et al.* (2010).

136 2.3 Data analysis

137 The data was log-transformed in order to meet the conditions of application of the parametric tests,
138 to reduce the effect of outliers skewing the data distribution, and to bring elemental concentrations
139 within the same range (Gobert *et al.*, 2017). Analyses were performed using XLSTAT software
140 (Addinsoft, 2019). A multivariate analysis of variance (MANOVA) was applied to explore the
141 influence of gender (2 levels), site (8 levels) and season (4 levels) factors to the observed
142 differences in TE concentration. MANOVA was then followed by posteriori univariate ANOVA
143 and post-hoc Tukey's honestly significant difference (HSD) tests. Pearson rank correlation tests
144 were performed to investigate the relationship between the trace element levels (inter-element
145 correlations) and the biological data (weight, size and gonadosomatic index). To determine the
146 significance and strength of each relationship, the correlation coefficient was calculated together
147 with p-values. A significant difference is considered as a p-value less than 0.05.

148 In order to compare the contamination levels of the different sites, the TE Pollution Index (TEPI)
149 was calculated for each site. Developed by Richir and Gobert (2014), the TEPI is a modified version
150 of the Metal Pollution Index (Usero *et al.*, 1996). It has the advantage of taking into account non-
151 metallic TEs to study As and Se. Moreover, unlike the Metal Pollution Index, the TEPI allows a
152 reliable comparison of study sites, regardless of TEs or the biological model used (Richir and
153 Gobert, 2014). As recommended for the calculation of the TEPI, the data were standardized by
154 mean normalization (Richir and Gobert, 2014). TEPI values were calculated using the following
155 formula: $TEPI = (Cf1 \square Cf2 \dots Cfn)^{1/n}$ where Cfn is the mean normalized concentration of the TE at
156 each site or station and n is the number of TE examined. The higher the TEPI value, the more
157 contaminated the site is. A 3-level water quality scale was established, using the method developed
158 by Richir *et al.* (2015). The first level corresponds to the Low Contamination Level (LCL), the
159 second level to a Medium Contamination Level (MCL) and the third level to a High Contamination
160 Level (HCL).

161 **3. Results**

162 3.1 Biotic factors of the sea urchin

163 The gonadosomatic index ranged from 0.72 to 6.7 and shows different patterns according to the
164 site considered (Figure 2). Among the 20 TEs measured, the concentration of 16 TEs is found
165 negatively correlated with gonadosomatic indices (Ag, Al, As, Ba, Cd, Co, Cr, Fe, Mn, Mo, Ni,
166 Pb, Sb, Se, U, V) (p-value < 0.05) and only one TE (Zn) is positively correlated with the
167 gonadosomatic indices (r = 0.011, p = 0.020).

168 There are significant positive correlations for 14 out of 20 TEs with both the weight and the size
169 of sea urchins. The larger the individuals, the more they tend to accumulate TEs (Ag, As, Cd, Co,
170 Cr, Fe, Li, Mo, Ni, Pb, Sb, Se, U, V) in the gonads (p-value < 0.05).

171 3.2 Trace element concentrations

172 The mean TE concentrations measured in the gonads of *Paracentrotus lividus*, at each site for all
173 seasons, are presented in Figure 2. Be and Bi observed concentrations are below the detection limit,
174 so they have not been taken into account in the statistical analyses. Zn is the most abundant TE
175 ($175.454 \pm 12.004 \text{ mg.kg}^{-1}$) while the lowest abundance is observed for Sn ($0.016 \pm 0.001 \text{ mg.kg}^{-1}$).
176 TE concentrations follow the sequence: Zn > Fe > As > Al > V > Cu > Se > Ni > Mn > Cr > U >
177 Co > Mo > Cd > Ba > Li > Ag > Pb > Sb > Sn. There are 14 significant negative correlations
178 (Ag - Sn, As - Cu, As - Sn, Cd - Cu, Cd - Sn, Zn - Cr, Zn - Fe, Zn - Mo, Zn - Ni, Zn - Pb, Zn - U,
179 Zn - V). The highest positive inter-elemental relationships are U-V (r = 0.83), Co-Cr (r = 0.78),
180 V - Cr (r = 0.71), Ni-Cr (r = 0.67) and U-Cr (r = 0.63) with p-value < 0.001.

181 The MANOVA results showed significant differences (p-value < 0.001) in TE concentrations
182 between the genders, the seasons and the sites.

183 3.3 Temporal variations of trace elements

184 The concentration of all TEs except Ag significantly vary among seasons variations
185 (p-value < 0.0001) (Table 1). Noteworthy, TE display similar fluctuation profiles that is a decrease
186 during the spring and summer seasons. The result of TEPI (Figure 3) is in accordance with such
187 results, evidencing that sea urchins have higher TE concentration during the legal harvesting season
188 (*i.e.* autumn and winter). Over the 8 values that are in HCL, 6 are in winter and concern four site
189 of three areas (*i.e.* Saint-Florent 1, Saint-Florent 2, Ajaccio 2, Calvi 1). The TE content is higher
190 when gonadosomatic indices are low in autumn and winter. Most TEs are probably stored in the
191 gonads and are not expelled with the gametes.

192 3.4 Spatial variations of trace elements

193 The TE concentrations vary considerably depending on the sampling stations (Table 2). Zn is the
194 only element that does not significantly vary spatially (p-value = 0.543). The annual TEPI reveals
195 two stations with High Contamination Level: Saint-Florent 1 (TEPI = 1.127) and Saint-Florent 2
196 (TEPI = 1.222). Conversely, Bonifacio 2 (TEPI = 0.726) and Calvi 2 (TEPI = 0.738) have been
197 classified as Low Contamination Level.

198 3.5 Variations of trace elements according to gender

199 The concentration of 11 out of the 20 TE are different between male and female (p-value < 0.05),
200 the average concentrations of Fe, Cr, Mo, Ni, Pb, U and V being higher in male, although those of
201 As, Cd, Se and Zn are higher in female (Table 3). Specifically, Zn and Fe are the two TEs presenting
202 the highest differences among gender, Zn being 5 times more concentrated in female and Fe 1.75
203 times more concentrated in males (p-value < 0.0001). The differences observed don't vary with the
204 seasons and very little according to the sites.

205

Discussion

206 Measurements of 22 TEs were conducted in 480 sea urchins. *Paracentrotus lividus* has a greater
207 tendency to accumulate essential TEs such as Cu, Fe, Mn or Zn in contrast to non-essential TEs
208 (e.g. Storelli *et al.*, 2001; Guendouzi *et al.*, 2017). The difference of TE concentration in sea urchin
209 gonads between the two genders has been little explored (e.g. Bayed *et al.*, 2005; Soualili *et al.*,
210 2008). Zn is an essential element in gametogenesis (Unuma *et al.*, 2007), which explains the high
211 content found in the gonads Unuma *et al.* (2007). Ovogenesis requires greater amounts of Zn than
212 spermatogenesis, which is why concentrations are higher in females than in males. According to
213 Unuma *et al.* (2007), the Major Yolk Protein (MYP) transports the assimilated Zn from the
214 digestive tract to the gonads.

215 Although non-essential TE are expected to have no physiological role, we have evidence however,
216 differential accumulation of those non-essential TE between male and female. They probably have
217 a strong affinity with certain essential TEs and are thus bioaccumulated with them. When there are
218 several TEs in an environment, antagonistic or synergistic effects can indeed occur (Kabata-
219 Pendias and Pendias, 2001). Moreover, positive inter-elemental relationships have been observed:
220 Cr-V; Cr-Ni; Cr-U are among the strongest combinations. Ni, U and V could be bioaccumulated
221 with Cr, an essential element, in male gonads. A high level of some non-essential TEs can reduce
222 sperm fertility or increase the frequency of embryo malformations that can lead to death (Pagano
223 *et al.*, 1986; Soualili *et al.*, 2008).

224 The population in Corsica increases considerably during summer with intense maritime and
225 recreational activities (INSEE, 2018). It's therefore surprising that the contamination levels are
226 lowest this season. Furthermore, studies indicate that transfers inside the echinoid occur on a
227 relatively short time scale (typically of the order of a week) (e.g. Miramand *et al.*, 1982; Warnau
228 *et al.*, 1996); These low concentrations of trace element in summer cannot be due to delays in gonad

229 contamination. An increase in the gonad weight of *Paracentrotus lividus* is correlated with a
230 decrease in TE concentrations, and inversely. There are two main cell populations in the germinal
231 epithelium of the sea urchin gonad: germinal cells and somatic cells called nutritive phagocytes
232 (Holland and Holland, 1969; Byrne, 1990; Walker *et al.*, 2005). Assuming that the TEs accumulate
233 mainly in the somatic cells and not in the germinal cells (Sellem and Guillou, 2007), a dilution of
234 the TEs is observed during gametogenesis and a concentration during spawning (Guendouzi *et al.*,
235 2017). The spawning period varies by site and is influenced by environmental factors such as
236 temperature, depth, photoperiod, quality and abundance of food (Fenaux, 1968; Byrne, 1990;
237 Lozano *et al.*, 1995; Guettaf, 1997). The higher gonad weight is probably due to gametogenesis
238 and its decrease to potential spawning. TE concentration tend to decrease in spring and summer
239 when gonadosomatic indices are highest and increase in autumn and winter when spawning has
240 occurred.

241 The size of sea urchin gonads is not necessarily related to the progress of gametogenesis alone.
242 They also grow because somatic cells, the nutritive phagocytes, store extensive nutrient reserves
243 before gametogenesis begins (Böttger *et al.*, 2004). Before the formation of gametes, nutritive
244 phagocytes could store reserves and accumulate TEs, which explains the significant positive
245 correlation between the concentration of Zn and the gonadosomatic index. About 80% of the total
246 proteins in the gonads, at the stage of pre-gametogenesis and the renewal of nutritive phagocytes
247 after spawning, are in both genders MYP, the protein involved in the transport of Zn in the gonads
248 (Unuma *et al.*, 2003; Unuma *et al.*, 2007). It is also worth noting that the TE concentration is higher
249 during the autumn, the period characterized by the refilling of nutrient reserves in the nutritive
250 phagocytes (Walker *et al.*, 2013). This supports the hypothesis that TEs are accumulated in nutritive
251 phagocytes.

252 The temporal variations in TEs concentration are related to the sea urchin's physiological
253 variations. Thus, to monitor TE contamination in the sea urchin gonads, it would be more

254 appropriate to avoid the spawning period. It's also important to take into account the sex of
255 individuals because some TEs are naturally more abundant in males or females. The role of TEs in
256 sea urchins needs to be studied further in order to better interpret their dynamics and their potential
257 consequences for the species.

258 In addition to physiological factors, other parameters such as food may be involved in these
259 temporal variations. Nutritive phagocytes develop when individuals are well nourished during the
260 pre-gametogenesis and nutrient phagocyte replacement phase (Walker and Lesser, 1998).
261 According to Schlacher-Hoenlinger and Schlacher. (1998), several macrophyte species have levels
262 of some TEs which have a marked seasonality with lower values in summer and higher in winter.
263 Consumption of food containing higher TE levels in winter may amplify the bioaccumulation
264 process. Moreover, according to Nédélec (1982), *Paracentrotus lividus* shows an alternation of
265 feeding and fasting phases; the period of fasting or low consumption when the gonads are highly
266 developed (Leighton, 1968). Therefore, the low TE contamination of macrophytes, coupled with
267 the reduced consumption of sea urchins during the summer season, may explain the low
268 concentrations recorded in summer in this study.

269 Biotic factors are not sufficient to explain the TE temporal dynamics in sea urchin gonads. In the
270 Bonifacio area, for example, gonadosomatic index variations are not related to the TE content
271 recorded. Bioavailability is determined by physiological and biological characteristics but also by
272 external environmental conditions (Chapman, 2008). These conditions, such as temperature, pH,
273 oxygen content and salinity, vary with the seasons. They modify TE availability and contribute to
274 the temporal variability of TE concentrations in the gonads.

275 The weight and size of individuals influence the TE concentrations measured in the gonads of
276 *Paracentrotus lividus*. The assumption being that sea urchins feed more when they are large and
277 thus bioaccumulate more contaminants. The use of TEPI allows a reliable comparison of TE

278 contamination both locally and internationally (Wilkes *et al.*, 2017; Ternengo *et al.*, 2018). The
279 seasons affect the TE concentrations in the sea urchin gonads, so it is not surprising to note that the
280 contamination levels of the sites observed in Ternengo *et al.* (2018) in winter are different from the
281 results of the annual TEPI in the present study.

282 As indicated by Ternengo *et al.* (2018), Saint-Florent 2 has a High Contamination Level probably
283 due to the proximity of the asbestos mine and the influence of soil leaching (Andral *et al.*, 2004;
284 Galgani *et al.*, 2006; Kantin and Pergent-Martini, 2007). High content of Ag has been measured
285 each season and is responsible for the High Level Contamination observed at Saint-Florent 1. In
286 Corsica, the Ag content is very low and only reflects the background level of agriculture (Luy *et*
287 *al.*, 2012) but although this site is now protected, the old mining concessions rich in Ag close to
288 the site could explain this high concentration (Gauthier, 2011). Calvi 2, identified as having a High
289 Contamination Level in Ternengo *et al.* (2018) and localised near the water treatment plant of the
290 city, presents the lowest level of contamination in Corsica. This is probably due to a bias in winter
291 sampling when some sites have sea urchins at an advanced stage of the nutrient reserve process.
292 Thus, even if sea urchins are harvested in winter to avoid the period of gametogenesis, the results
293 should be interpreted carefully, taking into account the physiological state. It is preferable to
294 combine the TE analysis and histology in order to know the physiological stage of the sea urchins
295 and better assess the contamination. It is also necessary to consider these results carefully because
296 despite the analysis of a large number of TEs, many other contaminants are not taken into account.
297 To verify the accuracy of these results, it is of interest to compare them with other bioindicators
298 such as *Mytilus galloprovincialis* (Lamarck, 1819) or *Posidonia oceanica*. The TEs measured at
299 high concentrations at the different sites of this study were also observed in these bioindicators, in
300 particular for Saint-Florent 2 (*e.g.* Kantin and Pergent-Martini, 2007; Lafabrie *et al.*, 2007; Lafabrie
301 *et al.*, 2008; Richir *et al.*, 2015). High concentrations of Ag were observed in *Posidonia oceanica*
302 at Ile Rousse in Richir *et al.* (2015), certainly originating in the same type of source as for the

303 contamination at Saint-Florent 1. In view of these results, the sea urchin *Paracentrotus lividus*
304 proves to be a good bioindicator and could complement the use of other bioindicators. An
305 ecotoxicological study of this sea urchin would provide information on the contamination at sea
306 and on the influence of the trophic chain and substrates, while the mussel would determine the
307 contamination in the water column, and *Posidonia oceanica* the contamination on substrate and in
308 the first link of the trophic chain (Richir and Gobert, 2014). In addition, depending on the sampling
309 period, one or other of these bioindicators would be more suitable. The spawning period would be
310 avoided for the sea urchin and the mussel while it would be more interesting to study *Posidonia*
311 *oceanica* in the spring (Kantin *et al.*, 2015).

312 **Conclusion**

313 This study highlights, once again, the need to consider biotic factors (gender, reproductive activity)
314 and abiotic factors (physical and chemical characteristics of seawater or food) in the use of sea
315 urchins as bioindicators. Differences in concentration were observed, according to sex, for 11 TEs.
316 This would probably be due to gametogenesis and to antagonistic or synergistic effects between
317 TEs. It is necessary to extend our knowledge to assess the effects of these contaminants on the sea
318 urchin populations. There are temporal variations marked by higher TE concentrations in autumn
319 and winter and, conversely, lower concentrations during the summer season. A dilution effect is
320 observed on the TE content in the gonads during gametogenesis. The use of gonads should be
321 avoided during the spawning period in order to avoid biased comparisons. A difference in
322 concentration of TEs is observed between the different sites. Due to the influence of the soil and
323 the former asbestos mine at Canari, the Saint-Florent area is the most contaminated. On the basis
324 of these results, *Paracentrotus lividus* appears as an interesting tool for achieving a better
325 understanding of anthropic pressures. Associated with other bioindicators such as *Mytilus*
326 *galloprovincialis* or *Posidonia oceanica*, the study of its ecotoxicological properties would enable
327 managers to act at source and reduce degradation or improve the ecological quality of water bodies.
328 In addition, it seems that no previous study of the sea urchin, taking all these factors into account,
329 has analyzed so many TEs in a Mediterranean region. These results are therefore essential and can
330 serve as a reference state for the Mediterranean sea.

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534

Table Caption

535 Table 1. Mean (mg.kg^{-1} dw \pm SE) trace elements concentrations in the gonad of *Paracentrotus*
536 *lividus* according to the season. abcd Dissimilar letters denote significant differences between
537 groups (p-value <0.05). p-value: $<0.05^*$; $<0.01^{**}$; $<0.001^{***}$ (**2 column fitting image**)

538 Table 2. Mean (mg.kg^{-1} dw \pm SE) trace elements concentrations (mg.kg^{-1} dry wt) in *Paracentrotus*
539 *lividus* from 8 stations in Corsica Island (A: Ajaccio; B: Bonifacio; C: Calvi; SF: Saint-Florent; 1:
540 reference site; 2: impacted site). abcd Dissimilar letters denote significant differences between
541 groups (p-value <0.05). p-value: $<0.05^*$; $<0.01^{**}$; $<0.001^{***}$ (**2 column fitting image or full**
542 **page**)

543 Table 3. Mean concentrations (mg.kg^{-1} dw \pm SE) of the 20 trace elements in the male and female
544 gonads of *Paracentrotus lividus*. P-value: $<0.05^*$; $<0.01^{**}$; $<0.001^{***}$ (**1,5 column fitting image**)

545 Table 1.

	Winter	Spring	Summer	Autumn	p-value
<i>Ag</i>	0.112 ± 0.018 ^a	0.159 ± 0.072 ^a	0.353 ± 0.101 ^a	0.187 ± 0.037 ^a	0.694
<i>Al</i>	32.888 ± 3.722 ^a	16.608 ± 1.757 ^b	9.637 ± 0.954 ^c	22.936 ± 2.802 ^a	<0.0001***
<i>As</i>	37.788 ± 1.845 ^a	32.083 ± 1.723 ^a	52.576 ± 3.061 ^b	53.253 ± 2.685 ^b	<0.0001***
<i>Ba</i>	0.305 ± 0.026 ^a	0.238 ± 0.034 ^b	0.116 ± 0.008 ^c	0.380 ± 0.092 ^a	<0.0001***
<i>Cd</i>	0.239 ± 0.019 ^a	0.240 ± 0.020 ^a	0.285 ± 0.022 ^{ab}	0.344 ± 0.027 ^b	0.0120*
<i>Co</i>	0.370 ± 0.042 ^a	0.295 ± 0.025 ^a	0.302 ± 0.024 ^a	0.504 ± 0.043 ^b	<0.0001***
<i>Cr</i>	1.204 ± 0.226 ^a	0.891 ± 0.101 ^a	0.850 ± 0.084 ^a	1.533 ± 0.193 ^b	<0.0001***
<i>Cu</i>	3.893 ± 0.068 ^a	2.949 ± 0.085 ^b	1.869 ± 0.041 ^c	2.731 ± 0.091 ^b	<0.0001***
<i>Fe</i>	92.708 ± 12.112 ^a	60.204 ± 4.489 ^{ab}	52.695 ± 3.371 ^b	117.273 ± 12.784 ^c	<0.0001***
<i>Li</i>	0.330 ± 0.008 ^a	0.239 ± 0.010 ^b	0.131 ± 0.004 ^c	0.324 ± 0.014 ^a	<0.0001***
<i>Mn</i>	2.264 ± 0.104 ^a	1.033 ± 0.060 ^b	0.568 ± 0.034 ^c	1.281 ± 0.101 ^d	<0.0001***
<i>Mo</i>	0.343 ± 0.014 ^a	0.311 ± 0.021 ^a	0.241 ± 0.022 ^b	0.346 ± 0.032 ^a	<0.0001***
<i>Ni</i>	1.735 ± 0.808 ^a	0.748 ± 0.157 ^a	0.549 ± 0.073 ^a	2.143 ± 0.335 ^b	<0.0001***
<i>Pb</i>	0.212 ± 0.036 ^a	0.131 ± 0.009 ^b	0.109 ± 0.008 ^b	0.223 ± 0.026 ^a	<0.0001***
<i>Sb</i>	0.169 ± 0.026 ^a	0.049 ± 0.006 ^b	0.036 ± 0.003 ^c	0.074 ± 0.004 ^d	<0.0001***
<i>Se</i>	1.990 ± 0.066 ^a	1.680 ± 0.057 ^b	1.627 ± 0.041 ^b	2.082 ± 0.069 ^a	<0.0001***
<i>Sn</i>	0.019 ± 0.002 ^a	0.016 ± 0.002 ^a	0.009 ± 0.001 ^b	0.020 ± 0.003 ^a	<0.0001***
<i>U</i>	0.991 ± 0.080 ^a	0.838 ± 0.067 ^a	0.872 ± 0.071 ^a	1.508 ± 0.125 ^b	<0.0001***
<i>V</i>	3.093 ± 0.217 ^a	3.133 ± 0.269 ^a	3.353 ± 0.289 ^a	5.108 ± 0.366 ^b	<0.0001***
<i>Zn</i>	210.167 ± 24.076 ^a	112.792 ± 14.137 ^b	144.450 ± 14.711 ^{bc}	234.408 ± 35.264 ^{ac}	<0.0001***

546

547 Table 2.

	A1	A2	B1	B2	C1	C2	SF1	SF2	P-value
Ag	0.043 ± 0.012 ^a	0.107 ± 0.015 ^{bc}	0.062 ± 0.008 ^b	0.015 ± 0.002 ^d	0.169 ± 0.054 ^c	0.146 ± 0.025 ^c	0.942 ± 0.234 ^e	0.138 ± 0.027 ^{bc}	<0.0001***
Al	41.522 ± 6.972 ^a	21.145 ± 2.824 ^b	19.512 ± 3.052 ^b	17.248 ± 2.354 ^b	9.102 ± 1.670 ^c	6.583 ± 0.746 ^c	19.105 ± 2.632 ^b	29.918 ± 4.144 ^{ab}	<0.0001***
As	31.895 ± 2.094 ^a	27.492 ± 3.123 ^b	42.410 ± 2.960 ^c	25.287 ± 1.427 ^{ab}	52.453 ± 3.243 ^{cd}	43.078 ± 2.578 ^c	71.095 ± 4.842 ^e	57.690 ± 2.935 ^{de}	<0.0001***
Ba	0.268 ± 0.036 ^{abc}	0.471 ± 0.182 ^a	0.223 ± 0.039 ^{abc}	0.247 ± 0.024 ^{ab}	0.189 ± 0.026 ^{bc}	0.212 ± 0.028 ^{abc}	0.304 ± 0.061 ^{abc}	0.161 ± 0.023 ^c	<0.0001***
Cd	0.123 ± 0.009 ^a	0.136 ± 0.016 ^a	0.246 ± 0.024 ^b	0.113 ± 0.008 ^a	0.339 ± 0.035 ^{cd}	0.314 ± 0.025 ^{bd}	0.491 ± 0.037 ^e	0.453 ± 0.039 ^{ce}	<0.0001***
Co	0.188 ± 0.014 ^a	0.216 ± 0.019 ^a	0.321 ± 0.026 ^{bc}	0.240 ± 0.020 ^{ac}	0.274 ± 0.033 ^{ac}	0.209 ± 0.017 ^a	0.395 ± 0.026 ^b	1.100 ± 0.078 ^d	<0.0001***
Cr	0.500 ± 0.052 ^a	0.505 ± 0.050 ^a	0.878 ± 0.088 ^b	0.688 ± 0.087 ^{ab}	0.854 ± 0.106 ^{ab}	0.657 ± 0.068 ^{ab}	1.301 ± 0.102 ^c	3.572 ± 0.515 ^d	<0.0001***
Cu	3.129 ± 0.170 ^{ab}	2.954 ± 0.143 ^{ab}	2.841 ± 0.107 ^{ab}	3.432 ± 0.166 ^a	2.536 ± 0.119 ^b	2.881 ± 0.118 ^{ab}	2.525 ± 0.120 ^b	2.586 ± 0.124 ^b	<0.0001***
Fe	76.417 ± 7.730 ^{ab}	98.113 ± 11.639 ^a	61.560 ± 5.072 ^{abc}	63.030 ± 8.361 ^{bc}	44.018 ± 4.256 ^c	60.570 ± 7.676 ^{bc}	55.663 ± 5.514 ^{bc}	186.388 ± 28.712 ^d	<0.0001***
Li	0.219 ± 0.017 ^a	0.250 ± 0.015 ^{abc}	0.250 ± 0.014 ^{abc}	0.272 ± 0.015 ^{bc}	0.258 ± 0.021 ^{abc}	0.307 ± 0.018 ^c	0.249 ± 0.015 ^{abc}	0.245 ± 0.021 ^{ab}	0.002**
Mn	1.463 ± 0.165 ^{ab}	1.305 ± 0.126 ^{ab}	1.149 ± 0.081 ^{ab}	1.263 ± 0.110 ^{ab}	0.965 ± 0.077 ^a	1.077 ± 0.097 ^a	1.019 ± 0.082 ^a	2.050 ± 0.243 ^b	<0.0001***
Mo	0.243 ± 0.034 ^{ab}	0.220 ± 0.020 ^b	0.371 ± 0.029 ^c	0.257 ± 0.026 ^{ab}	0.418 ± 0.052 ^{cd}	0.248 ± 0.018 ^{abd}	0.419 ± 0.039 ^c	0.306 ± 0.024 ^{acd}	<0.0001***
Ni	0.516 ± 0.112 ^a	0.729 ± 0.121 ^{ab}	0.492 ± 0.051 ^{ab}	0.583 ± 0.084 ^{ab}	0.983 ± 0.274 ^{ab}	0.510 ± 0.069 ^a	0.824 ± 0.088 ^b	5.712 ± 1.662 ^c	<0.0001***
Pb	0.191 ± 0.046 ^{abcd}	0.243 ± 0.070 ^{abc}	0.188 ± 0.013 ^{ab}	0.248 ± 0.029 ^a	0.129 ± 0.011 ^{cde}	0.110 ± 0.008 ^{de}	0.138 ± 0.013 ^{bcde}	0.102 ± 0.008 ^e	<0.0001***
Sb	0.077 ± 0.010 ^{abc}	0.072 ± 0.016 ^{ab}	0.135 ± 0.030 ^c	0.076 ± 0.011 ^{ab}	0.049 ± 0.004 ^a	0.051 ± 0.004 ^a	0.092 ± 0.013 ^{bc}	0.104 ± 0.043 ^{ab}	<0.0001***
Se	1.661 ± 0.066 ^{ab}	1.516 ± 0.071 ^a	2.039 ± 0.096 ^{bc}	1.827 ± 0.092 ^{abc}	1.831 ± 0.080 ^{bc}	1.799 ± 0.073 ^{abc}	2.001 ± 0.084 ^{bc}	2.086 ± 0.108 ^c	<0.0001***
Sn	0.011 ± 0.001 ^a	0.015 ± 0.001 ^b	0.036 ± 0.003 ^c	0.034 ± 0.006 ^c	0.007 ± 0.001 ^d	0.011 ± 0.003 ^{ad}	0.008 ± 0.001 ^{ad}	0.006 ± 0.001 ^d	<0.0001***
U	0.692 ± 0.100 ^a	0.652 ± 0.065 ^a	1.078 ± 0.102 ^{bc}	0.660 ± 0.083 ^a	1.335 ± 0.170 ^b	0.818 ± 0.095 ^{ac}	1.784 ± 0.168 ^d	1.398 ± 0.137 ^{bd}	<0.0001***
V	2.570 ± 0.365 ^a	2.647 ± 0.265 ^a	4.294 ± 0.412 ^b	2.657 ± 0.405 ^a	3.775 ± 0.437 ^{ab}	2.483 ± 0.263 ^a	6.419 ± 0.526 ^c	4.531 ± 0.397 ^{bc}	<0.0001***
Zn	161.450 ± 25.331 ^a	179.567 ± 27.477 ^a	243.450 ± 59.087 ^a	134.083 ± 19.116 ^a	123.133 ± 23.404 ^a	248.133 ± 42.577 ^a	181.800 ± 28.013 ^a	132.017 ± 26.079 ^a	0.543

	Males	Females	p-value
<i>Ag</i>	0.124 ± 0.021	0.260 ± 0.055	0.086
<i>Al</i>	22.983 ± 2.229	18.725 ± 1.605	0.123
<i>As</i>	36.082 ± 1.487	49.624 ± 1.828	<0.0001***
<i>Ba</i>	0.299 ± 0.057	0.231 ± 0.016	0.837
<i>Cd</i>	0.215 ± 0.013	0.322 ± 0.017	<0.0001***
<i>Co</i>	0.372 ± 0.028	0.365 ± 0.023	0.647
<i>Cr</i>	1.234 ± 0.136	1.036 ± 0.101	0.010**
<i>Cu</i>	2.950 ± 0.075	2.796 ± 0.065	0.085
<i>Fe</i>	106.575 ± 9.597	61.933 ± 3.994	<0.0001***
<i>Li</i>	0.266 ± 0.010	0.249 ± 0.008	0.341
<i>Mn</i>	1.250 ± 0.077	1.313 ± 0.064	0.652
<i>Mo</i>	0.370 ± 0.022	0.267 ± 0.012	<0.0001***
<i>Ni</i>	1.741 ± 0.490	0.968 ± 0.151	<0.0001***
<i>Pb</i>	0.204 ± 0.017	0.143 ± 0.016	<0.0001***
<i>Sb</i>	0.096 ± 0.016	0.072 ± 0.004	0.273
<i>Se</i>	1.542 ± 0.033	2.065 ± 0.043	<0.0001***
<i>Sn</i>	0.018 ± 0.002	0.015 ± 0.001	0.731
<i>U</i>	1.163 ± 0.084	0.972 ± 0.051	0.009**
<i>V</i>	4.088 ± 0.248	3.369 ± 0.184	<0.0001***
<i>Zn</i>	53.287 ± 8.275	264.223 ± 18.069	<0.0001***

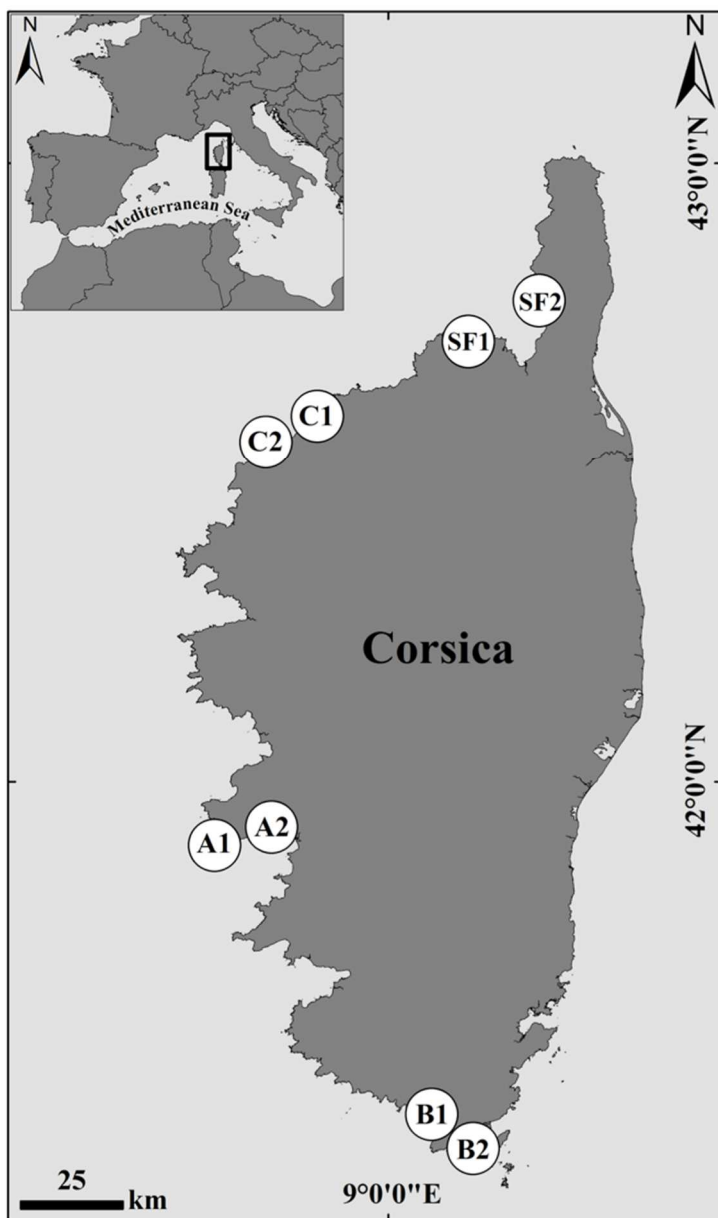
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Figure Caption

552 Figure 1. Location of study coastal areas in Corsica (NW Mediterranean. France), showing
553 the eight sampling stations of *Paracentrotus lividus* and their characterization. **(1 column**
554 **fitting image)**

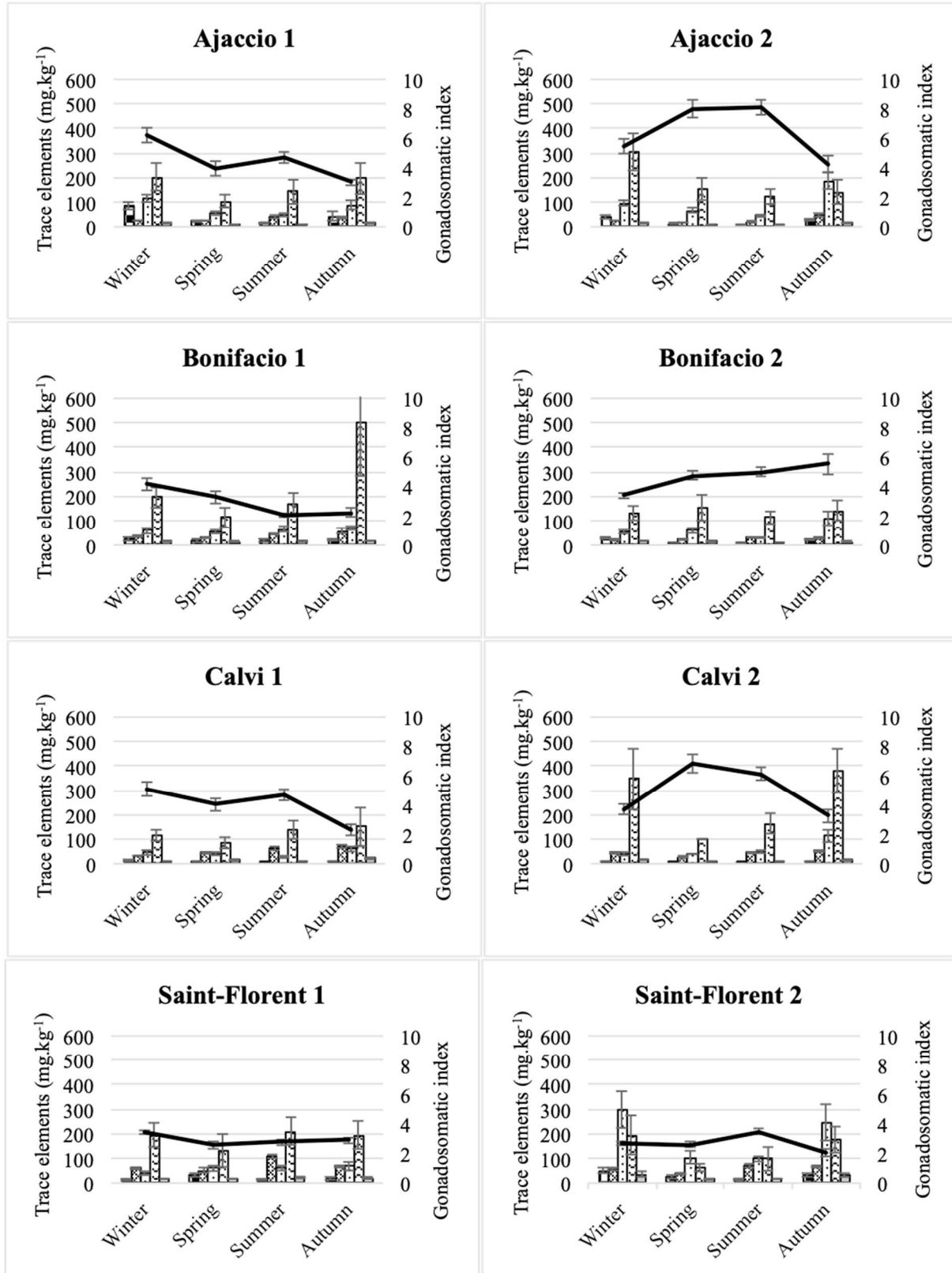
555 Figure 2. Variation in mean (mg.kg⁻¹ dw ± SE) trace elements concentrations in the gonads of
556 *Paracentrotus lividus* and gonadosomatic indices according to the season and on the eight sites
557 (1: reference site; 2: impacted site). **(2 column fitting image)**

558 Figure 3. Trace Element Pollution Index (TEPI) variation of the eight sites (A: Ajaccio; B:
559 Bonifacio; C: Calvi; SF: Saint-Florent; 1: reference site; 2: impacted site) according to the
560 season (HCL: High Contamination Level, MCL: Medium Contamination Level, LCL: Low
561 Contamination Level). **(1,5 column fitting image)**



Sampling site	Anthropogenic sources identified
Ajaccio 1 (A1)	Reference station
Ajaccio 2 (A2)	Wastewater treatment plant
Bonifacio 1 (B1)	Reference station
Bonifacio 2 (B2)	Commercial harbor / marina
Calvi 1 (C1)	Reference station
Calvi 2 (C2)	Wastewater treatment plant
Saint-Florent 1 (SF1)	Reference station
Saint-Florent 2 (SF2)	Former asbestos mine

564 Figure 2.

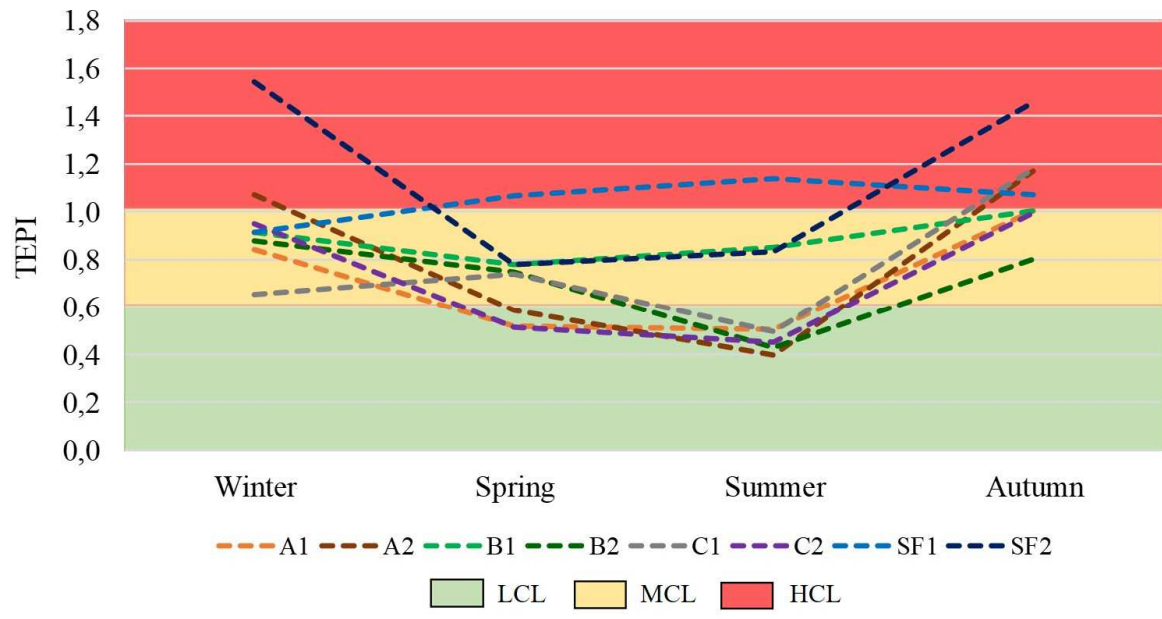


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568 Figure 3.



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