

# Seasonal change in trace element concentrations of Paracentrotus lividus: Its use as a bioindicator

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O. El Idrissi, M. Marengo, A. Aiello, S. Gobert, V. Pasqualini, et al.. Seasonal change in trace element concentrations of Paracentrotus lividus: Its use as a bioindicator. Ecological Indicators, 2020, 112, pp.106063 -. 10.1016/j.ecolind.2019.106063 . hal-03489972

# HAL Id: hal-03489972 https://hal.science/hal-03489972

Submitted on 7 Mar 2022

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2	use as a bioindicator				
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15					
16	Highlights				
17 18	- Importance physiological factors have been identified				
19 20	- Gonadosomatic index is the variable most closely related to temporal variations				
21 22	- Trace elements contamination is lower during the summer season				
23	- The use of gonads should be avoided during the spawning period (autumn/winter)				

#### 24 Abstract

An assessment of classical and emerging trace element contamination was conducted on gonads of 25 the sea urchin Paracentrotus lividus (Lamarck, 1819), in Corsica (Western Mediterranean). The 26 27 aim of this study was to evaluate the contamination levels at different sites by following the seasonal variation of 22 trace elements. The sea urchins analyzed were taken in 2017 from reference 28 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 additional support for other tools for the diagnosis and monitoring of coastal water quality. It 34 35 provides useful new data to enable managers to act at the source and reduce degradation in order to improve the ecological quality of marine waters. 36

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

#### 38 **1. Introduction**

The growth of industrial, agricultural and urban activities gives rise to the introduction of 39 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 (Nordberg et al., 2007; Amiard, 2011). Furthermore, intensive human activities, particularly in 42 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 of sewage, 129,000 tons of mineral oil, 60,000 tons of mercury, 3,800 tons of lead and 36,000 tons 45 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 a vulnerable ecological unit (Turley, 1999). Furthermore, as the water bodies renews with a few 48 49 decades in the Mediterranean versus a few centuries for the ocean, this sea is a veritable laboratory for observing the pressures and changes that humans exert on the environment (Bethoux et al., 50 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 environment, they can remain in solution, be adsorbed on sedimentary particles, precipitate to the 56 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., 1989; Walter et al., 1989) and for human consumers of sea urchin gonads. As a result of the threats 61

posed by TEs in the environment, they must be continuously monitored from their emission sources
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, ease of harvesting, longevity, relative sedentarity and high tolerance of pollutants, Paracentrotus 69 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 the most trace elements (Augier et al., 1989), thus, the study of their ecotoxicological properties is 72 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 environmental (season) factors (Warnau et al., 1998; Guendouzi et al., 2017; Rocha et al., 2019). 75 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).

In the Mediterranean, numerous studies using Paracentrotus lividus as a bioindicator have studied 78 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 ecological importance (Lawrence and Sammarco, 1982; Kelly, 2004). The sea urchin is of high 82 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 and more particularly of macrophyte communities (Lawrence and Sammarco, 1982). 86

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

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

#### 2. Material and Methods

#### 100 2.1 <u>Sampling sites, collection and preparation of samples</u>

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.

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

# 116 2.2 <u>Trace element analysis</u>

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

method described by Richir and Gobert (2014). A total of 22 trace elements were analyzed: silver 123 (Ag), aluminium (Al), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), 124 125 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 Reference Materials (CRM), DORM-4 (fish protein), NIST 1566b (oyster) and NIST 2976 (mussel 129 130 tissue). For each TE, detection limit (LD) and quantification limit (LQ) were calculated according to Currie (1999) and Grinzaid et al. (1977) depending on their specific blank distribution. The 131 132 results are expressed in milligrams of element per kilogram of dry weight ± standard error (mg.kg<sup>-</sup> <sup>1</sup> dw  $\pm$  se). TEs with values generally below the detection limit were removed from the database. 133 For the others, concentrations below the LD were replaced with a value of LD/2, as reported by 134 135 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) was calculated for each site. Developed by Richir and Gobert (2014), the TEPI is a modified version 149 of the Metal Pollution Index (Usero et al., 1996). It has the advantage of taking into account non-150 151 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 mean normalization (Richir and Gobert, 2014). TEPI values were calculated using the following 154 formula:  $TEPI = (Cf1 \quad Cf2...Cfn)^{1/n}$  where Cfn is the mean normalized concentration of the TE at 155 each site or station and n is the number of TE examined. The higher the TEPI value, the more 156 157 contaminated the site is. A 3-level water quality scale was established, using the method developed by Richir et al. (2015). The first level corresponds to the Low Contamination Level (LCL), the 158 second level to a Medium Contamination Level (MCL) and the third level to a High Contamination 159 160 Level (HCL).

161 **3. Results** 

### 162 3.1 <u>Biotic factors of the sea urchin</u>

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

There are significant positive correlations for 14 out of 20 TEs with both the weight and the size of sea urchins. The larger the individuals, the more they tend to accumulate TEs (Ag, As, Cd, Co, 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  $(175.454 \pm 12.004 \text{ mg.kg}^{-1})$  while the lowest abundance is observed for Sn  $(0.016 \pm 0.001 \text{ mg.kg}^{-1})$ . 175 176 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 (Ag - Sn, As - Cu, As - Sn, Cd - Cu, Cd - Sn, Zn - Cr, Zn - Fe, Zn - Mo, Zn - Ni, Zn - Pb, Zn - U, 178 179 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  $\leq 0.001$ .

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

# 3.3 Temporal variations of trace elements

The concentration of all TEs except Ag significantly vary among seasons variations 184 (p-value < 0.0001) (Table 1). Noteworthy, TE display similar fluctuation profiles that is a decrease 185 186 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 <u>Spatial variations of trace elements</u>

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

## 198 3.5 Variations of trace elements according to gender

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

#### 205 Discussion

Measurements of 22 TEs were conducted in 480 sea urchins. Paracentrotus lividus has a greater 206 207 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 et al., 1986; Soualili et al., 2008). 223

The population in Corsica increases considerably during summer with intense maritime and recreational activities (INSEE, 2018). It's therefore surprising that the contamination levels are lowest this season. Furthermore, studies indicate that transfers inside the echinoid occur on a relatively short time scale (typically of the order of a week) (e.g. Miramand *et al.*, 1982; Warnau *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 epithelium of the sea urchin gonad: germinal cells and somatic cells called nutritive phagocytes 231 232 (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 nutrient phagocytes 247 after spawning, are in both genders MYP, the protein involved in the transport of Zn in the gonads (Unuma et al., 2003; Unuma et al., 2007). It is also worth noting that the TE concentration is higher 248 249 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 appropriate to avoid the spawning period. It's also important to take into account the sex of individuals because some TEs are naturally more abundant in males or females. The role of TEs in sea urchins needs to be studied further in order to better interpret their dynamics and their potential consequences for the species.

258 In addition to physiological factors, other parameters such as food may be involved in these temporal variations. Nutritive phagocytes develop when individuals are well nourished during the 259 260 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 feeding and fasting phases; the period of fasting or low consumption when the gonads are highly 265 266 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.

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

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

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

To verify the accuracy of these results, it is of interest to compare them with other bioindicators such as *Mytilus galloprovencialis* (Lamarck, 1819) or *Posidonia oceanica*. The TEs measured at high concentrations at the different sites of this study were also observed in these bioindicators, in particular for Saint-Florent 2 (*e.g.* Kantin and Pergent-Martini, 2007; Lafabrie *et al.*, 2007; Lafabrie *et al.*, 2008; Richir *et al.*, 2015). High concentrations of Ag were observed in *Posidonia oceanica* 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 proves to be a good bioindicator and could complement the use of other bioindicators. An 304 305 ecotoxicological study of this sea urchin would provide information on the contamination at sea and on the influence of the trophic chain and substrates, while the mussel would determine the 306 307 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).

#### Conclusion

This study highlights, once again, the need to consider biotic factors (gender, reproductive activity) 313 314 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 concentration of TEs is observed between the different sites. Due to the influence of the soil and 322 323 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 galloprovencialis 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. In addition, it seems that no previous study of the sea urchin, taking all these factors into account, 328 329 has analyzed so many TEs in a Mediterranean region. These results are therefore essential and can 330 serve as a reference state for the Medirerranean sea.

#### 331 Acknowledgments

332 This work was supported by the University of Corsica (France) and the University of Liege (Belgium). This study would not have been possible without the financial support of the French 333 334 Government, the Collectivity of Corsica and the MISTRALS (Mediterranean Integrated STudies 335 at Regional And Local Scales) research program that authors are grateful for their cooperation. 336 This work is part of the STARECAPMED (STAtion of Reference and rEsearch on Change of local and global Anthropogenic Pressures on Mediterranean Ecosystems Drifts) project. The authors are 337 338 grateful to the scuba diver team of the UMS 3514 Stella Mare for their efforts during the underwater 339 sampling and for providing the necessary facilities. The authors wish to thank M. Paul for proof-

reading the manuscript. This publication has the MARE publication number MARE393.

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# **Table Caption**

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

538 Table 2. Mean (mg.kg<sup>-1</sup> dw  $\pm$  SE) trace elements concentrations (mg.kg-1dry 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**)

534

543 Table 3. Mean concentrations (mg.kg<sup>-1</sup> 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**)

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

547	Table	2.

	A1	A2	<b>B1</b>	B2	C1	C2	SF1	SF2	P-value
Ag	$0.043 \pm 0.012^{a}$	$0.107 \pm 0.015^{\rm bc}$	$0.062 \pm 0.008^{b}$	$0.015 \pm 0.002^{d}$	$0.169 \pm 0.054^{\rm c}$	$0.146 \pm 0.025^{\rm c}$	$0.942 \pm 0.234^{e}$	$0.138 \pm 0.027^{\rm bc}$	<0.0001***
Al	$41.522 \pm 6.972^{a}$	$21.145 \pm 2.824^{b}$	$19.512 \pm 3.052^{b}$	$17.248 \pm 2.354^{b}$	$9.102 \pm 1.670^{\rm c}$	$6.583 \pm 0.746^{\rm c}$	$19.105 \pm 2.632^{b}$	$29.918 \pm 4.144^{ab}$	<0.0001***
As	$31.895 \pm 2.094^{a}$	$27.492 \pm 3.123^{b}$	$42.410 \pm 2.960^{\circ}$	25.287 ± 1.427 <sup>ab</sup>	$52.453 \pm 3.243^{cd}$	$43.078 \pm 2.578^{\circ}$	$71.095 \pm 4.842^{e}$	$57.690 \pm 2.935^{de}$	<0.0001***
Ba	$0.268 \pm 0.036^{abc}$	$0.471 \pm 0.182^{a}$	$0.223 \pm 0.039^{abc}$	$0.247 \pm 0.024^{ab}$	$0.189 \pm 0.026^{bc}$	$0.212 \pm 0.028^{abc}$	$0.304 \pm 0.061^{abc}$	$0.161 \pm 0.023^{c}$	<0.0001***
Cd	$0.123 \pm 0.009^{a}$	$0.136 \pm 0.016^{a}$	$0.246 \pm 0.024^{b}$	$0.113 \pm 0.008^{a}$	$0.339 \pm 0.035^{cd}$	$0.314 \pm 0.025^{bd}$	$0.491 \pm 0.037^{e}$	$0.453 \pm 0.039^{ce}$	<0.0001***
Со	$0.188 \pm 0.014^{a}$	$0.216 \pm 0.019^{a}$	$0.321 \pm 0.026^{\rm bc}$	$0.240 \pm 0.020^{\rm ac}$	$0.274 \pm 0.033^{\rm ac}$	$0.209 \pm 0.017^{a}$	$0.395 \pm 0.026^{b}$	$1.100 \pm 0.078^{d}$	<0.0001***
Cr	$0.500 \pm 0.052^{a}$	$0.505 \pm 0.050^{a}$	$0.878 \pm 0.088^{b}$	$0.688 \pm 0.087^{ab}$	$0.854 \pm 0.106^{ab}$	$0.657 \pm 0.068^{ab}$	$1.301 \pm 0.102^{\rm c}$	$3.572 \pm 0.515^{d}$	<0.0001***
Си	$3.129 \pm 0.170^{ab}$	$2.954 \pm 0.143^{ab}$	$2.841 \pm 0.107^{ab}$	$3.432 \pm 0.166^{a}$	$2.536 \pm 0.119^{b}$	$2.881 \pm 0.118^{ab}$	$2.525 \pm 0.120^{b}$	$2.586 \pm 0.124^{b}$	<0.0001***
Fe	76.417 ± 7.730 <sup>ab</sup>	98.113 ± 11.639 <sup>a</sup>	$61.560 \pm 5.072^{abc}$	$63.030 \pm 8.361^{bc}$	$44.018 \pm 4.256^{\rm c}$	$60.570 \pm 7.676^{bc}$	55.663 ± 5.514 <sup>bc</sup>	$186.388 \pm 28.712^{d}$	<0.0001***
Li	$0.219 \pm 0.017^{a}$	$0.250 \pm 0.015^{abc}$	$0.250 \pm 0.014^{abc}$	$0.272 \pm 0.015^{\rm bc}$	$0.258 \pm 0.021^{abc}$	$0.307 \pm 0.018^{\rm c}$	$0.249 \pm 0.015^{abc}$	$0.245 \pm 0.021^{ab}$	0.002**
Mn	$1.463 \pm 0.165^{ab}$	$1.305 \pm 0.126^{ab}$	$1.149 \pm 0.081^{ab}$	$1.263 \pm 0.110^{ab}$	$0.965 \pm 0.077^{a}$	$1.077 \pm 0.097^{a}$	$1.019 \pm 0.082^{a}$	$2.050 \pm 0.243^{b}$	<0.0001***
Мо	$0.243 \pm 0.034^{ab}$	$0.220 \pm 0.020^{b}$	$0.371 \pm 0.029^{\rm c}$	$0.257 \pm 0.026^{ab}$	$0.418 \pm 0.052^{cd}$	$0.248 \pm 0.018^{abd}$	$0.419 \pm 0.039^{\rm c}$	$0.306 \pm 0.024^{acd}$	<0.0001***
Ni	$0.516 \pm 0.112^{a}$	$0.729 \pm 0.121^{ab}$	$0.492 \pm 0.051^{ab}$	$0.583 \pm 0.084^{ab}$	$0.983 \pm 0.274^{ab}$	$0.510 \pm 0.069^{a}$	$0.824 \pm 0.088^{b}$	$5.712 \pm 1.662^{c}$	<0.0001***
Pb	$0.191 \pm 0.046^{abcd}$	$0.243 \pm 0.070^{abc}$	$0.188 \pm 0.013^{ab}$	$0.248 \pm 0.029^{a}$	$0.129 \pm 0.011^{cde}$	$0.110 \pm 0.008^{de}$	$0.138 \pm 0.013^{bcde}$	$0.102 \pm 0.008^{\text{e}}$	<0.0001***
Sb	$0.077 \pm 0.010^{abc}$	$0.072 \pm 0.016^{ab}$	$0.135 \pm 0.030^{\rm c}$	$0.076 \pm 0.011^{ab}$	$0.049 \pm 0.004^{a}$	$0.051 \pm 0.004^{a}$	$0.092 \pm 0.013^{bc}$	$0.104 \pm 0.043^{ab}$	<0.0001***
Se	$1.661 \pm 0.066^{ab}$	$1.516 \pm 0.071^{a}$	$2.039 \pm 0.096^{bc}$	$1.827 \pm 0.092^{abc}$	$1.831 \pm 0.080^{\rm bc}$	$1.799 \pm 0.073^{abc}$	$2.001 \pm 0.084^{bc}$	$2.086 \pm 0.108^{\circ}$	<0.0001***
Sn	$0.011 \pm 0.001^{a}$	$0.015 \pm 0.001^{b}$	$0.036 \pm 0.003^{\circ}$	$0.034 \pm 0.006^{\rm c}$	$0.007 \pm 0.001^{d}$	$0.011 \pm 0.003^{ad}$	$0.008 \pm 0.001^{ad}$	$0.006 \pm 0.001^{d}$	<0.0001***
U	$0.692 \pm 0.100^{a}$	$0.652 \pm 0.065^{a}$	$1.078 \pm 0.102^{bc}$	$0.660 \pm 0.083^{a}$	$1.335 \pm 0.170^{b}$	$0.818 \pm 0.095^{\rm ac}$	$1.784 \pm 0.168^{d}$	$1.398 \pm 0.137^{bd}$	<0.0001***
V	$2.570 \pm 0.365^{a}$	$2.647 \pm 0.265^{a}$	$4.294 \pm 0.412^{b}$	$2.657 \pm 0.405^{a}$	$3.775 \pm 0.437^{ab}$	$2.483 \pm 0.263^{a}$	$6.419 \pm 0.526^{\rm c}$	$4.531 \pm 0.397^{bc}$	<0.0001***
Zn	$161.450 \pm 25.331^{a}$	$179.\overline{567 \pm}$ 27.477 <sup>a</sup>	$243.\overline{450 \pm}$ 59.087 <sup>a</sup>	$134.083 \pm 19.116^{a}$	$123.\overline{133 \pm} 23.404^{a}$	$248.133 \pm 42.577^{a}$	$181.800 \pm 28.013^{a}$	$132.017 \pm 26.079^{a}$	0.543

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

549 Table 3.

551	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)
555 556 557	Figure 2. Variation in mean (mg.kg-1 dw $\pm$ SE) trace elements concentrations in the gonads of <i>Paracentrotus lividus</i> and gonadosomatic indices according to the season and on the eight sites (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)







