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

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ABSTRACT

An assessment of classical and emerging trace element contamination was conducted on gonads of the sea urchin *Paracentrotus lividus* (Lamarck, 1819), in Corsica (Western Mediterranean). The 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 and more impacted sites in four Corsican areas. The results obtained shown the importance of biotic factors such as gender, reproduction and the way of life. Variations have been highlighted with lower trace element concentrations during the summer season. This is mainly due to a dilution phenomenon resulting from gametogenesis. The pollution index (TEPI) was determined and 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 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.

1. Introduction

The growth of industrial, agricultural and urban activities gives rise to the introduction of considerable amounts of chemicals in marine coastal ecosystems. These substances have toxic 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 coastal areas, have a major environmental impact on these productive zones (Papathanassiou and Gabrielides, 1999). The United Nations Environment Programme estimated that 650 million tons of sewage, 129000 tons of mineral oil, 60000 tons of mercury, 3800 tons of lead and 36000 tons of phosphates are dumped into the Mediterranean each year. In addition, 70 per cent of the 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 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., 1999).

Trace elements (TEs) are among the most common contaminants in the marine ecosystem. Due to their toxicity, persistence and ability to accumulate in marine organisms, they are considered as serious pollutants in marine ecosystems (Bonanno and Di Martino, 2017). TEs are present in the 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 bottom, or be bioaccumulated or biomagnified by organisms and to reach concentrations that can be toxic (Warnau et al., 1998). Above a certain threshold, all TEs present a potential danger that can cause disturbances at cellular level, individual level, and also population or ecosystem levels (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 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).

In order to assess the levels of contaminants available in the ecosystem, organisms can be used as bioindicators. Postmetamorphic echinoids in general, and *Paracentrotus lividus* (Lamarck, 1816), in particular, are interesting candidates for the bioindication of TEs contaminations and have already been used in the Mediterranean and elsewhere (*e.g.* Augier et al., 1989; Guendouzi et al., 2017; Ternengo

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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 lividus* is an organism that is recognized for its role as bioindicator (Warnau et al., 1998; Geraci et 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 also of public health interest (Salvo et al., 2015). Trace elements concentration in the gonads is 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). The elementary constitution of the sea urchin reflects the composition of its environment and 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 classical TEs such as zinc or lead, but there has been little or no description of the contamination related to emerging trace elements (*e.g.* Rouane-Hacene et al., 2017; Guendouzi et al., 2017). Well-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 commercial value and represents a complementary resource for artisanal fishing. The taste quality of its gonads also makes it a species appreciated and targeted by recreational fishing. In addition, 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).

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.

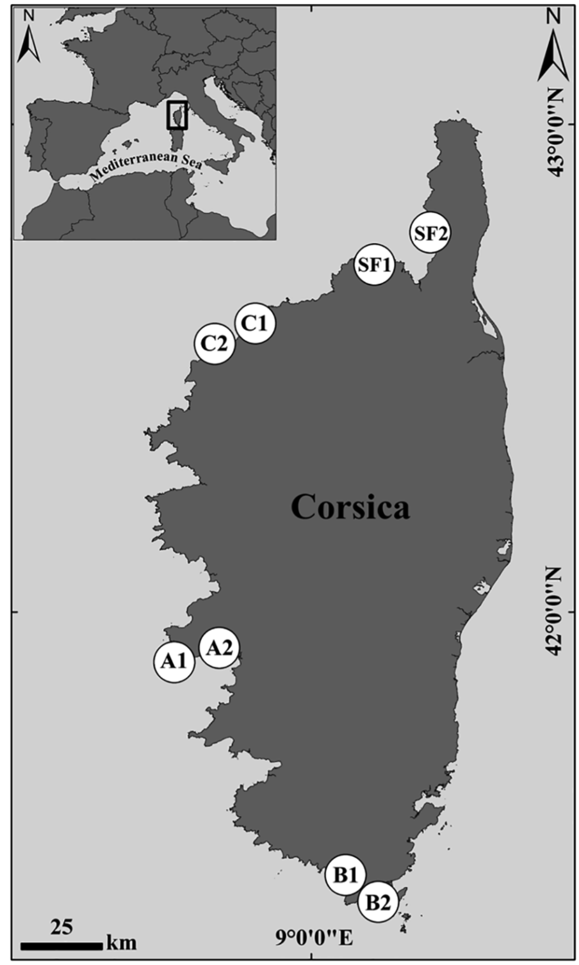
The purpose of this study is (i) to monitor the spatio-temporal dynamics of 22 TEs (classical and emerging) in sea urchin gonads collected along the Corsican coasts, and (ii) to evaluate the seasonal patterns of change in the pollution index characterizing each site to determine whether these variations 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

2.1. Sampling sites, collection and preparation of samples

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



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

Fig. 1. Location of study coastal areas in Corsica (NW Mediterranean. France), showing the eight sampling stations of *Paracentrotus lividus* and their characterization.

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.

2.2. Trace element analysis

Prior to the analysis, samples were lyophilized (CHRIST LCG Lyochamber Guard 121550 PMMA/Alpha 1-4 LD plus) and ground in

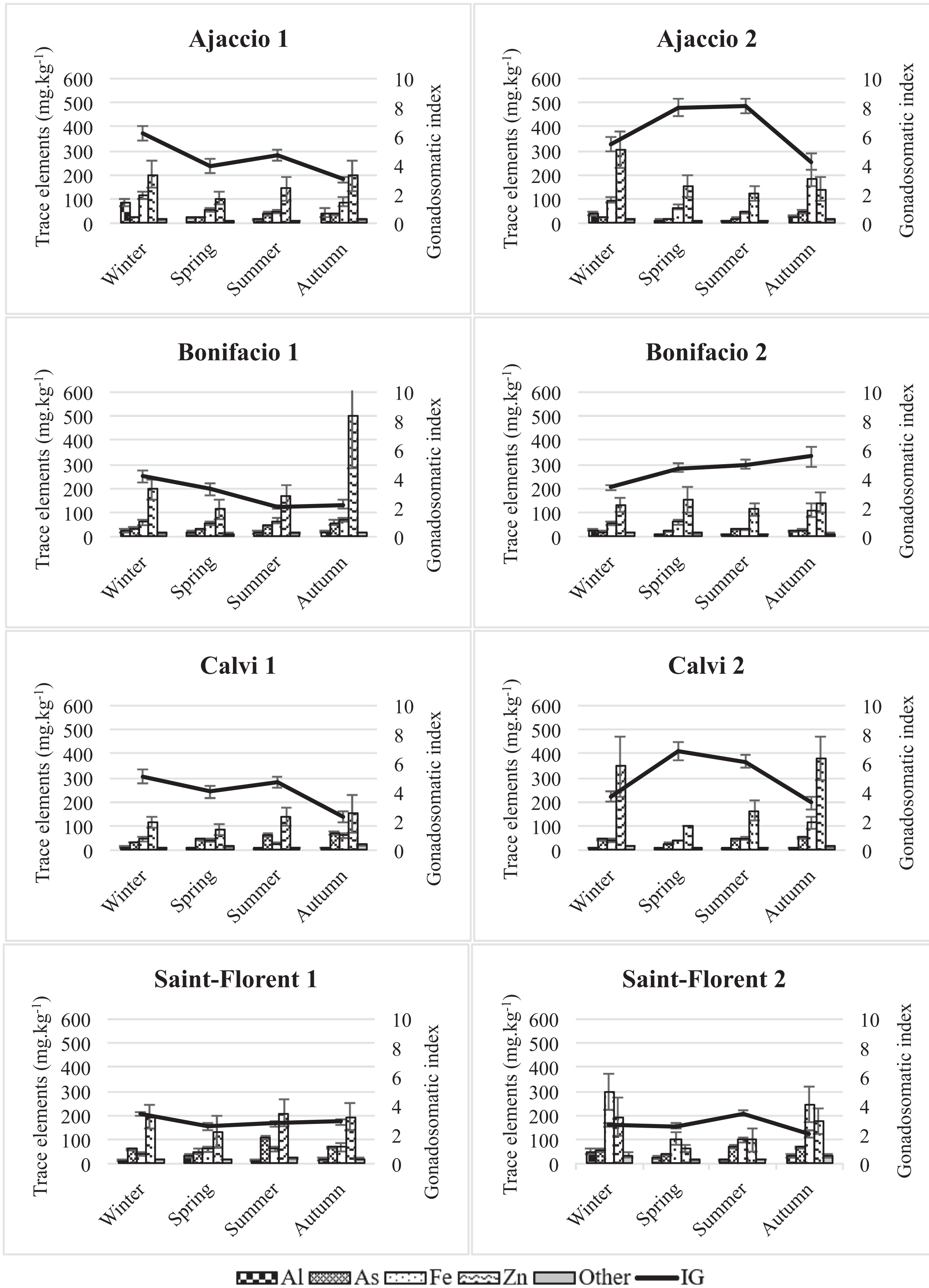


Fig. 2. Variation in mean (mg.kg^{-1} dw ± SE) trace elements concentrations in the gonads of *Paracentrotus lividus* and gonadosomatic indices according to the season and on the eight sites (1: reference site; 2: impacted site).

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 (Ag), aluminium (Al), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), uranium (U), vanadium (V), and zinc (Zn). In order to check the purity of the chemicals used, a large number of chemical 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 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 results are expressed in milligrams of element per kilogram of dry weight ± standard error ($mg.kg^{-1}$ dw ± SE). TEs with values generally below the detection limit were removed from the database. For the others, concentrations below the LD were replaced with a value of LD/2, as reported by Skrbić et al. (2010).

2.3. Data analysis

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

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 of the Metal Pollution Index (Usero et al., 1996). It has the advantage of taking into account non-metallic TEs to study As and Se. Moreover, unlike the Metal Pollution Index, the TEPI allows a reliable comparison of study sites, regardless of TEs or the biological model used (Richir and 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 formula: $TEPI = (Cf1 * Cf2 \ldots Cfn)^{1/n}$ where Cfn is the mean normalized concentration of the TE at each site or station and n is the number of TE examined. The higher the TEPI value, the more 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 second level to a Medium Contamination Level (MCL) and the third level to a High Contamination Level (HCL).

3. Results

3.1. Biotic factors of the sea urchin

The gonadosomatic index ranged from 0.72 to 6.7 and shows different patterns according to the site considered (Fig. 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).

3.2. Trace element concentrations

The mean TE concentrations measured in the gonads of *Paracentrotus lividus*, at each site for all seasons, are presented in Fig. 2. Be and Bi observed concentrations are below the detection limit, so they have not been taken into account in the statistical analyses. Zn is the most abundant TE (175.454 ± 12.004 $mg.kg^{-1}$) while the lowest abundance is observed for Sn (0.016 ± 0.001 $mg.kg^{-1}$). TE concentrations follow the sequence: Zn > Fe > As > Al > V > Cu > Se > Ni > Mn > Cr > U > 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, Zn – V). The highest positive inter-elemental relationships are U-V ($r = 0.83$), Co-Cr ($r = 0.78$), V – Cr ($r = 0.71$), Ni-Cr ($r = 0.67$) and U-Cr ($r = 0.63$) with p-value < 0.001.

The MANOVA results showed significant differences (p-value < 0.001) in TE concentrations 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 (p-value < 0.0001) (Table 1). Noteworthy, TE display similar fluctuation profiles that is a decrease during the spring and summer seasons. The result of TEPI (Fig. 3) is in accordance with such results, evidencing that sea urchins have higher TE concentration during the legal harvesting season (i.e. autumn and winter). Over the 8 values that are in HCL, 6 are in winter and concern four site of three areas (i.e. Saint-Florent 1, Saint-Florent 2, Ajaccio 2, Calvi 1). The TE content is higher when gonadosomatic indices are low in autumn and winter. Most TEs are probably stored in the gonads and are not expelled with the gametes.

3.4. Spatial variations of trace elements

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.

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.

4. Discussion

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

Table 1
Mean ($mg.kg^{-1}$ dw ± 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***.

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

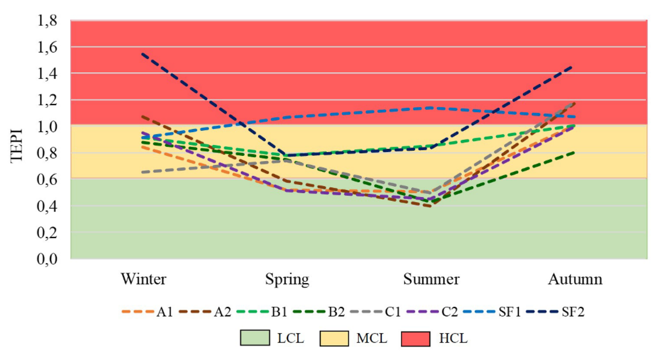


Fig. 3. Trace Element Pollution Index (TEPI) variation of the eight sites (A: Ajaccio; B: Bonifacio; C: Calvi; SF: Saint-Florent; 1: reference site; 2: impacted site) according to the season (HCL: High Contamination Level, MCL: Medium Contamination Level, LCL: Low Contamination Level).

Major Yolk Protein (MYP) transports the assimilated Zn from the digestive tract to the gonads.

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

The population in Corsica increases considerably during summer with intense maritime and recreational activities (INSEE, 2018). It is 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 contamination. An increase in the gonad weight of *Paracentrotus lividus* is correlated with a 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 (Holland and Holland, 1969; Byrne, 1990; Walker et al., 2005). Assuming that the TEs accumulate mainly in the somatic cells and not in the germinal cells (Sellem and Guillou, 2007), a dilution of the TEs is observed during gametogenesis and a concentration during spawning (Guendouzi et al., 2017). The spawning period varies by site and is influenced by environmental factors such as temperature, depth, photoperiod, quality and abundance of food (Fenaux, 1968; Byrne, 1990; Lozano et al., 1995; Guettaf, 1997). The higher gonad weight is probably due to gametogenesis and its decrease to potential spawning. TE concentration tend to decrease in spring and summer when gonadosomatic indices are highest and increase in autumn and winter when spawning has occurred.

The size of sea urchin gonads is not necessarily related to the progress of gametogenesis alone. They also grow because somatic cells, the nutritive phagocytes, store extensive nutrient reserves before gametogenesis begins (Böttger et al., 2004). Before the formation of gametes, nutritive phagocytes could store reserves and accumulate TEs, which explains the significant positive correlation between the concentration of Zn and the gonadosomatic index. About 80% of the total proteins in the gonads, at the stage of pre-gametogenesis and the renewal of nutrient phagocytes 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 during the autumn, the period characterized by the refilling of nutrient reserves in the nutritive phagocytes (Walker et al., 2013). This supports the hypothesis that TEs are accumulated in nutritive phagocytes.

The temporal variations in TEs concentration are related to the sea urchin's physiological 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.

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 pre-gametogenesis and nutrient phagocyte replacement phase (Walker and Lesser, 1998). According to Schlacher-Hoenlinger and Schlacher. (1998), several macrophyte species have levels of some TEs which have a marked seasonality with lower values in summer and higher in winter.

Table 2
Mean ($mg.kg^{-1}$ dw ± SE) trace elements concentrations ($mg.kg^{-1}$ dw) in *Paracentrotus lividus* from 8 stations in Corsica Island (A: Ajaccio; B: Bonifacio; C: Calvi; SF: Saint-Florent; 1: reference site; 2: impacted site). abcd Dissimilar letters denote significant differences between groups (p-value < 0.05). p-value: < 0.05*; < 0.01**; < 0.001***.

	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

Table 3
Mean concentrations ($mg.kg^{-1}$ dw ± SE) of the 20 trace elements in the male and female gonads of *Paracentrotus lividus*. p-value: < 0.05*; < 0.01**; < 0.001***.

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

Consumption of food containing higher TE levels in winter may amplify the bioaccumulation 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 developed (Leighton, 1968). Therefore, the low TE contamination of macrophytes, coupled with the reduced consumption of sea urchins during the summer season, may explain the low 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 availability 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.

As indicated by Ternengo et al. (2018), Saint-Florent 2 has a High Contamination Level probably due to the proximity of the asbestos mine and the influence of soil leaching (Andral et al., 2004; Galgani et al., 2006; Kantin and Pergent-Martini, 2007). High content of Ag has been measured each season and is responsible for the High Level Contamination observed at Saint-Florent 1. In Corsica, the Ag content is very low and only reflects the background level of agriculture (Luy et al., 2012) but although this site is now protected, the old mining concessions rich in Ag close to the site could explain this high concentration (Gauthier, 2011). Calvi 2, identified as having a High Contamination Level in Ternengo et al. (2018) and localised near the water treatment plant of the city, presents the lowest level of contamination in Corsica. This is probably due to a bias in winter sampling when some sites have sea urchins at an advanced stage of the nutrient reserve process. Thus, even if sea urchins are harvested in winter to avoid the period of gametogenesis, the results should be interpreted carefully, taking into account the physiological state. It is preferable to combine the TE analysis and histology in order to know the physiological stage of the sea urchins and better assess the contamination. It is also necessary to consider these results carefully because 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 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 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 contamination in the water column, and *Posidonia oceanica* the contamination on substrate and in the first link of the trophic chain (Richir and Gobert, 2014). In addition, depending on the sampling period, one or other of these bioindicators would be more suitable. The spawning period would be avoided for the sea urchin and the mussel while it would be more interesting to study *Posidonia oceanica* in the spring (Kantin et al., 2015).

5. Conclusion

This study highlights, once again, the need to consider biotic factors (gender, reproductive activity) and abiotic factors (physical and chemical characteristics of seawater or food) in the use of sea urchins as bioindicators. Differences in concentration were observed, according to sex, for 11 TEs. This would probably be due to gametogenesis and to antagonistic or synergistic effects between TEs. It is necessary to extend our knowledge to assess the effects of these contaminants on the sea urchin populations. There are temporal variations marked by higher TE concentrations in autumn and winter and, conversely, lower concentrations during the summer season. A dilution effect is observed on the TE content in the gonads during gametogenesis. The use of gonads should be 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 the former asbestos mine at Canari, the Saint-Florent area is the most contaminated. On the basis of these results, *Paracentrotus lividus* appears as an interesting tool for achieving a better understanding of anthropic pressures. Associated with other bioindicators such as *Mytilus galloprovencialis* or *Posidonia oceanica*, the study of its ecotoxicological properties would enable 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, has analyzed so many TEs in a Mediterranean region. These results are therefore essential and can serve as a reference state for the Medirerranean sea.

CRediT authorship contribution statement

O. El Idrissi: Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **M. Marengo:** Formal analysis, Writing - review & editing. **A. Aiello:** Funding acquisition, Resources. **S. Gobert:** Writing - review & editing, Funding acquisition, Resources. **V. Pasqualini:** Writing - review & editing, Funding acquisition, Resources. **S. Ternengo:** Investigation, Writing - review & editing, Funding acquisition, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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