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Elemental fingerprinting of sea urchin (*Paracentrotus lividus*) gonads to assess food safety and trace its geographic origin

Renato Mamede[a,*], Irina A. Duarte[a], Isabel Caçador[a,b], Susanne E. Tanner[a,c], Marisa Silva[a,b], David Jacinto[d], Vanessa F. Fonseca[a,c], Bernardo Duarte[a,b,**]

[a] *MARE—Marine and Environmental Sciences Centre & ARNET – Aquatic Research Infrastructure Network Associated Laboratory, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal*

[b] *Departamento de Biologia Vegetal da Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal*

[c] *Departamento de Biologia Animal da Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal*

[d] *MARE—Marine and Environmental Sciences Centre & ARNET – Aquatic Research Infrastructure Network Associated Laboratory, Laboratório de Ciências do Mar, Universidade de Évora, Sines, Portugal*

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ABSTRACT

Sea urchin gonads are an economically valuable seafood item, considered a delicacy in many parts of the world. However, its consumption can either pose a food safety threat, as they accumulate potentially toxic elements from contaminated environments or promote the depletion of natural stocks due to the high demand. Knowing their harvesting location is therefore paramount to guarantee food safety and the conservation of natural stocks. In this study, the elemental fingerprints of sea urchin (*Paracentrotus lividus*) gonads collected in several locations along the Portuguese coast, in 2020 and 2021, were used to assess the levels of some potentially toxic elements (i.e., As, Cu, Pb and Zn) and to trace the harvesting location of this marine resource. *P. lividus* gonads presented mean levels of As, Cu, Pb and Zn above the thresholds recommended for human consumption (considering mollusks due to the absence of safety thresholds for echinoderms), in at least one of the sampling locations. The elemental fingerprints of gonads hold the potential to trace the harvesting location of *P. lividus*, even when it was used elemental fingerprints of different years to train and test the models.

1. Introduction

Seafood consumption worldwide has grown in the last decades (FAO, 2020). Along with its high economic value, seafood is considered an important component of a healthy diet (FAO, 2020). The gonads (or roe) of several sea urchin species are an example of a seafood product that are both economically valuable, due to be considered a delicacy, and highly nutritive (Archana and Babu, 2016; Lawrence, 2007). Among the most consumed sea urchin species in Europe, *Paracentrotus lividus* presents a distribution from the Northeast Atlantic (from Scotland to south Morocco) to the Mediterranean Sea (Boudouresque and Verlaque, 2013; Stefánsson et al., 2017).

Sea urchins accumulate the elements present in the environment, particularly in their gonads, which allows their use as bioindicators of environmental contamination (Soualili et al., 2008). This can pose either a benefit for consumers, as they accumulate essential elements (Ternengo et al., 2018), or a food safety threat if the harvesting location is contaminated with high levels of potentially toxic elements (e.g., As, Cu, Pb and Zn), as these can negatively affect human health (Barchiesi et al., 2020; Bielmyer et al., 2012). The sources of such elements in the environment can be natural (e.g., rock erosion) or anthropogenic (e.g., industry and domestic effluents) (Richir and Gobert, 2016), with sea urchins accumulating these elements both directly from the surrounding seawater or through their diet (Ahn et al., 2009).

Due to the increasing consumers' demand, limited resources, high commercial value and the complex supply chains, seafood trading is very prone to fraud, which can threaten the sustainability of natural stocks, food safety of consumers and their fair valorization (Fonner and Sylvia, 2015; Fox et al., 2018; Leal et al., 2015). In fact, the number of mislabeling cases of seafood is escalating, aiming to associate it to

* Corresponding author.

** Corresponding author at: MARE—Marine and Environmental Sciences Centre & ARNET – Aquatic Research Infrastructure Network Associated Laboratory, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal.

E-mail addresses: rjmamede@fc.ul.pt (R. Mamede), baduarte@fc.ul.pt (B. Duarte).

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locations, fishing practices or species more reputable and/or economically more valuable (Luque and Donlan, 2019). Therefore, the development of tools to certify both the commercial designation of the species and its scientific name, as well as production method and place of harvesting is paramount (EC, 2002; EU, 2013; Leal et al., 2015).

Diverse biochemical tools have been applied in the geographic traceability of seafood, of which fatty acid profiling (Mamede et al., 2020; Ricardo et al., 2017a), stable isotope analysis (Gopi et al., 2019b; Ortea and Gallardo, 2015) and elemental fingerprinting (Duarte et al., 2022a; Varrà et al., 2021) are among the most commonly used (Gopi et al., 2019a). The rationale of using elemental fingerprinting to trace the geographic origin of seafood is based on the assumption that different chemical compositions of locations (i.e., water, rocks and food resources) are mirrored by the elemental composition of seafood tissues (Gopi et al., 2019a). Indeed, elemental fingerprints of several marine organisms have been used to trace their geographic origin, either using non-edible tissues (usually hard structures), such as bivalve shells (Bennion et al., 2019; Mamede et al., 2021), barnacle capitula (Albuquerque et al., 2016), and fish otoliths (Campana et al., 2000; Tanner et al., 2012), or the edible soft tissues, such as the sea cucumber muscle (Kang et al., 2018; Liu et al., 2012), fish muscle (Duarte et al., 2022a; Gopi et al., 2019b), shrimp muscle (Gopi et al., 2019c; Smith and Watts, 2009), and some tissues with fast regenerations as sea urchin gonads (e.g., cephalopods ink; Bua et al., 2017; Duarte et al., 2022b). The use of elemental fingerprints of edible tissues, such as sea urchin gonads, is particularly interesting as it allows to both directly evaluate the levels of potentially toxic elements and trace their provenance. Among the most used techniques of elemental fingerprinting, total reflection X-ray fluorescence spectrometry (TXRF) proved to be cost-effective, accurate, highly selective and sensitive (Duarte et al., 2022a; Gopi et al., 2019b; Machado et al., 2020).

Improving the cost-effectiveness of such traceability tools will allow tracing seafood in a faster and less expensive way, promoting, therefore, their implementation in real-case scenarios. Furthermore, temporal variability of seafood elemental fingerprints (Bennion et al., 2021; Morrison et al., 2019; Ricardo et al., 2017b) must be taken into account, as this can impair the traceability if the samples used to train and test the models were not collected over a short time period. Traceability studies have accounted by using samples for both training and test datasets that were collected at the same time (e.g., Bennion et al., 2019; Duarte et al., 2022a), however this can be very time consuming and costly due to the need of periodically sampling. Therefore, tracing the geographic origin of seafood using models previously parametrized can substantially improve the cost-effectiveness of these tools.

In this study, the elemental fingerprints of *P. lividus* gonads collected in several locations along the Portuguese coast in 2020 and in 2021 were analyzed pursuing three aims: i) evaluate levels of potentially toxic elements (i.e., As, Cu, Pb and Zn) in each of the locations; ii) trace harvesting locations of *P. lividus*, separately for each year and; iii) evaluate if the temporal variability of elemental fingerprints impairs geographic traceability when using temporally mismatched samples.

2. Material and methods

2.1. Study areas and sample collection

Sea urchins (*P. lividus*) were collected in tidal pools in four locations in November 2020 (Viana do Castelo, VC; Matosinhos, Ma; Lisbon, L; Sines, S) and in five locations in July 2021 (VC; Ma; L; S; Sagres, Sa) along the North Atlantic Portuguese coast (Fig. 1). During the sampling preparation, 8 sea urchin gonads per location were collected (except in L with 16 samples), making a total of 88 specimens (in 2020: 4 locations x 8 or 16 samples = 40 samples; in 2021: 5 locations x 8 or 16 samples = 48 samples). The number of individuals in site L, results from pooling of two closely related sites (L1 and L2, c.a. 27 km; Supplementary Fig. S1), following a preliminary statistical analysis, as described in Section 3.2. Specimens were transported fresh to the laboratory, where they were measured (average diameter ± standard deviation = 5.33 ± 0.64 cm), weighted (average weight ± standard deviation = 45.9 ± 7.84 g) and dissected with scissors and plastic tweezers to collect gonads for elemental analysis. No significant differences were observed between individuals collected at the different sampling sites for both weight and diameter, in any of the considered years (Kruskal-Wallis test, $p > 0.05$). The sex of the specimens was identified by direct observation, under a microscope, of released gametes from the dissected gonads for all individuals and a sex ratio 1:1 (males:females) was ensured, to the extent possible in all areas, to avoid bias. Dissected gonad samples were then stored at − 80 ºC, until further analysis.

2.2. Elemental analysis

Gonads were freeze-dried and digested with HNO_3 in Teflon reactors, using microwave digestion (Multiwave GO, Anton Paar GmbH, Graz, Austria) according to EPA 3052 method (EPA, 1996). An internal standard (Gallium, Ga, final concentration 1 mg•L^{-1}) was added to each sample. Then, 5 μL of each sample was applied and dried in a siliconized quartz disk (BrukerNano, Germany). Elemental concentrations (As, Br, Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Rb, S, Se, Sr, Ti, V, Y and Zn) were analyzed through Total Reflection X-ray Fluorescence spectroscopy (TXRF S2 PICOFOX, Bruker, Germany). Instrumental recalibration (gain correction, sensitivity analysis and multi-elemental standards) and analytical blanks were used for quality assurance and control. Elemental concentrations were determined by comparison with the internal standard (Duarte et al., 2022a) and extraction efficiency was confirmed through the analysis of International certified reference materials (ERM-CE278k Mussel) (Table 1).

2.3. Data and statistical analysis

For food safety purposes, international regulatory or recommended maximum levels in mollusks (used due to the absence of recommended levels for echinoderms) were used to compare with concentrations of potentially toxic elements in sea urchin gonads: As (1 mg•Kg^{-1}), Cu (30 mg•Kg^{-1}), Pb (1–2 mg•Kg^{-1}) and Zn (30 mg•Kg^{-1}) (EC, 2001; FAO, 1983; FSANZ, 2015).

Using the whole elemental profile, the elemental concentrations in sea urchin gonads were log (x + 1) transformed and Euclidean distances between samples were obtained. Then, to test if the elemental fingerprints of gonads can be used to trace the geographic origin of *P. lividus*, a Canonical Analysis of Principal Coordinates (CAP) was used separately for each year. CAP uses the intrinsic characteristics (elemental concentrations) of samples to classify them within previously defined groups (in this case harvesting locations), allocating the samples to the group whose centroid is closest to it (Anderson et al., 2008). Similarity Percentage (SIMPER) was then applied to evaluate the statistical average square distances (d^2) between groups of samples and the contribution of each variable to the separations of groups. To investigate the coherence among the elemental fingerprints of the gonads collected at different sampling sites, a preliminary analysis was performed, with both sampling years together.

To test if the temporal variability of elemental fingerprints of *P. lividus* gonads impairs the allocation of unknown samples using a model built with temporally mismatched samples, samples of 2021 (only from locations coincident with 2020) were introduced as a test dataset in the CAP model developed with samples collected in 2020. This was performed to mimic a real case scenario when it would be necessary to determine the geographic origin of *P. lividus* specimens without a model built with samples from the same year.

Violin plots, with probability density of the data smoothed by a kernel density estimator, and chord diagrams were elaborated in R, using respectively ggplot2 and circalize packages (Core Team, 2020). CAP and SIMPER were performed using PRIMER v6 with the add-on

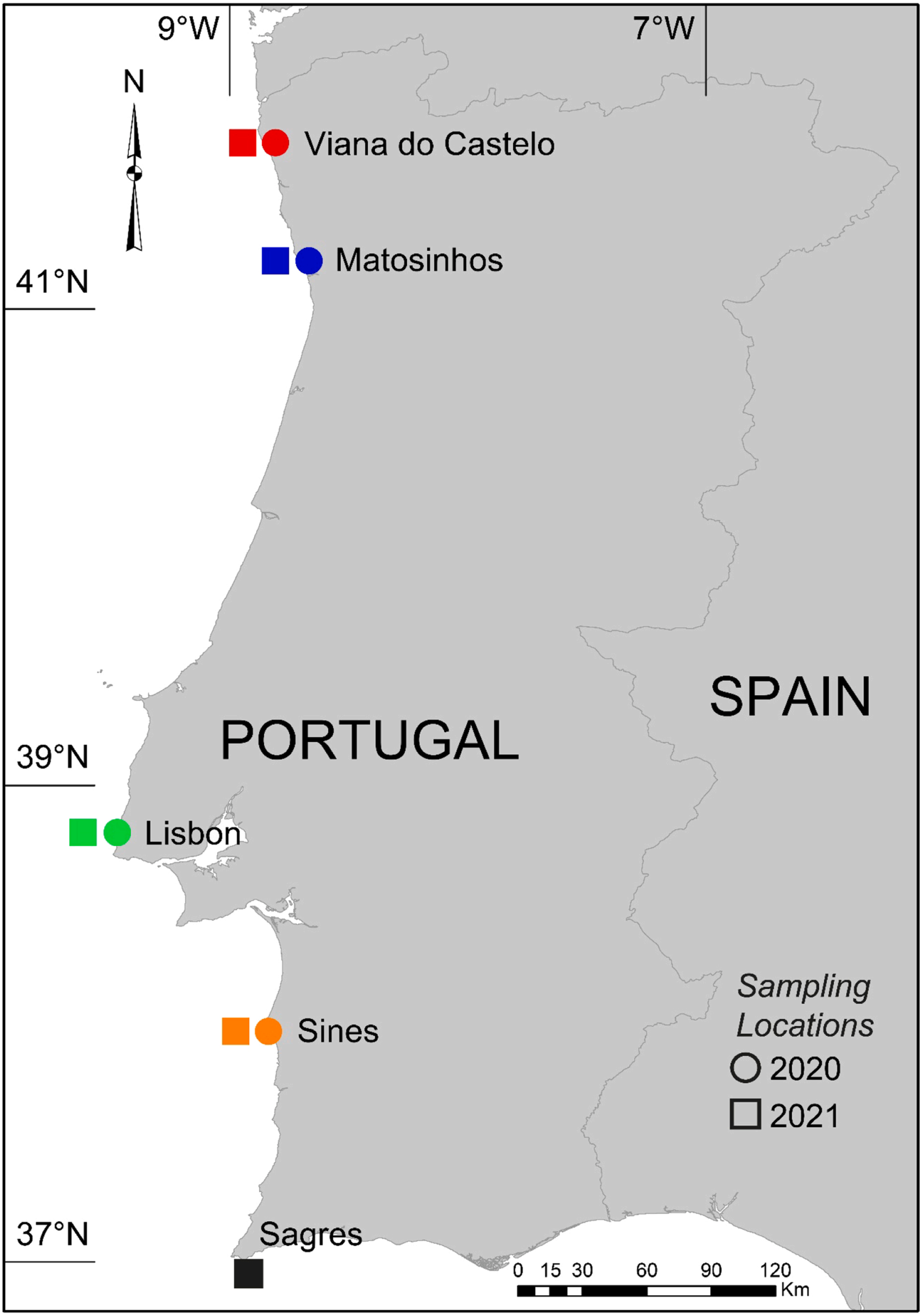

Fig. 1. Sampling locations of *Paracentrotugs lividus* along the Portuguese Atlantic coast (Viana do Castelo - VC; Matosinhos - Ma; Lisbon - L; Sines - S; Sagres - Sa). Circles: samples collected in 2020; Squares: samples collected in 2021.

Table 1
Mussel (ERM-CE278k) certified and analyzed elemental values, uncertainty (mg•Kg^{-1}) and calculated extraction efficiency (average ± standard deviation, N = 5).

Element	Certified value	Uncertainty	Measured value	Extraction Efficiency (%)
Cr	0.73	0.22	0.67 ± 0.10	91.3 ± 5.3
Mn	4.88	0.24	3.35 ± 0.10	68.6 ± 1.9
Fe	161.0	8.0	198.03 ± 0.67	123.0 ± 0.4
Ni	0.69	0.15	0.78 ± 0.05	113.1 ± 5.8
Cu	5.98	0.27	7.10 ± 0.07	118,8 ± 1.0
Zn	71.0	4.0	73.29 ± 0.28	103.2 ± 0.4
As	6.7	0.4	7.25 ± 0.07	108.2 ± 0.9
Se	1.62	0.12	1.51 ± 0.03	93.3 ± 1.9
Rb	2.46	0.16	2.45 ± 0.05	99.6 ± 1.9
Sr	19.0	0.0	18.55 ± 0.34	97.6 ± 1.8
Cd	0.336	0.025	0.32 ± 0.02	96.6 ± 4.5
Pb	2.18	0.18	2.47 ± 0.05	113.3 ± 2.1

PERMANOVA+ (Anderson et al., 2008; Clarke and Gorley, 2006). Chord diagrams were used to plot the distance among sample groups based on its similarity values (d^2) provided by SIMPER analysis.

3. Results

3.1. Seafood safety

The overall mean levels (mg•Kg^{-1} wet weight) of potentially toxic elements of both years ranged as follows: As (0.99–2.65), Cu (0.49–1.07), Pb (0–2.59) and Zn (40−142) (Fig. 2). Regarding the levels of As in *P. lividus* gonads, Ma presented the highest mean levels in both years and S (2021) was the sole location, presenting mean values (0.99 mg•Kg^{-1}) below those recommended (1 mg•Kg^{-1}) (Fig. 2). Nonetheless, despite presenting mean levels above the recommended limit, L (2020 and 2021), S (2020) and Sa (2021) also presented specimens with As levels below its recommended threshold. In contrast, the levels of Cu in all *P. lividus* gonads were below the maximum recommended (30 mg•Kg^{-1}, Fig. 2). Concerning Pb, *P. lividus* from S (2020) was the only group presenting mean levels that exceeded the three thresholds used (1, 1.5 and 2 mg•Kg^{-1}, Fig. 2), while S (2021) and Sa (2021) presented mean levels that surpassed that established for cephalopods (1 mg•Kg^{-1}, Fig. 2). Nonetheless, an accentuated decrease of Pb levels in samples from S between 2020 and 2021 was registered (Fig. 2). The mean levels of Zn of samples from VC (2020), L (2020) and S (2021) surpassed the set threshold (30 mg•Kg^{-1}, Fig. 2), being also important to emphasize a marked decrease in the mean levels of Zn in samples from S between 2020 and 2021.

3.2. Tracing geographic origin of P. lividus

The preliminary analysis of the elemental profiles of the individuals collected at the different sampling sites, in both sampling years, revealed a high degree of similarity between the samples from two closely related sites L1 and L2 (Supplementary Material Fig. S1), as shown by CAP plot (Supplementary Material Fig. S2) and respective confusion matrix (Supplementary Material Table S1). This analysis revealed a high degree of classification mismatch between both sites, reinforcing the resemblance of the elemental profiles of the individuals. This fact, allied to the geographical proximity of the two sites (c.a. 27 km), contributed to the

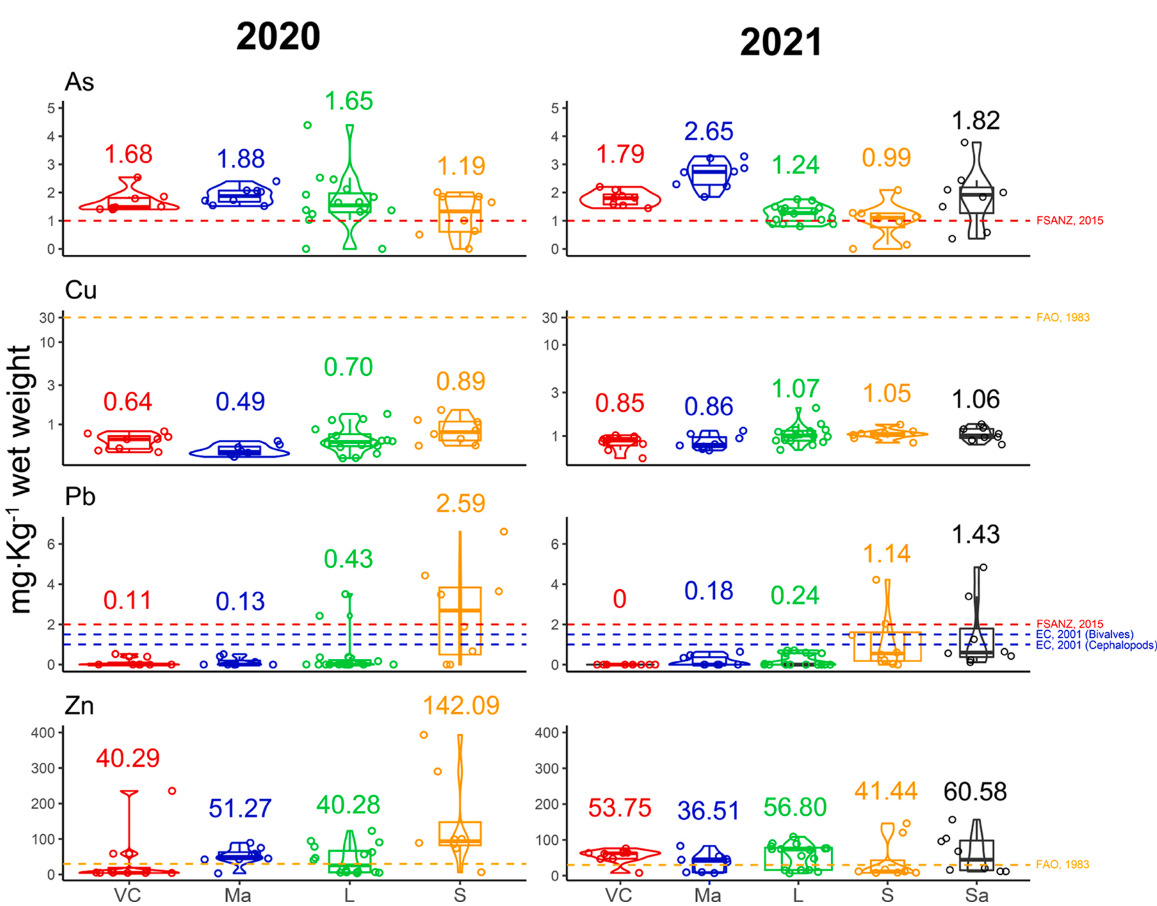

Fig. 2. Arsenic (As), copper (Cu), lead (Pb) and zinc (Zn) levels in *Paracentrotus lividus* gonads (mg•Kg^{-1} wet weight) collected in four locations in 2020 and five locations in 2021 along the Portuguese Atlantic coast (dotted red lines represent the safety threshold according to international regulatory authorities). Mean levels per location are presented above the respective violin plots and boxplots. Viana do Castelo - VC; Matosinhos - Ma; Lisbon - L; Sines - S; Sagres – Sa.

pooling of L1 and L2 into a single sampling site, hereafter denominated as L (Lisbon).

Elemental fingerprints of samples collected in 2020 presented an overall allocation success of 72.5 % (Table 2), confirming the good separation of samples by location (Fig. 3A). The accuracy in this classification was equal or above 50 % in all locations, namely 87.5 % for VC (2020), 75.0 % for Ma (2020), 50.0 % for L (2020) and 100.0 % for S (2020) (Table 2). The misclassified samples were, in general, allocated to locations sharing the lowest d^2 (Table 2, Fig. 4). For instance, the misclassification of samples from VC (2020) to L (2020), or the misclassification of Ma (2020) to VC (2020), are justified by the lower average square Euclidean distances (d^2) between these locations (Table 2, Fig. 4). Regarding the CAP based on samples collected in 2021, the overall classification success presented was 66.7 %, also with values higher than or equal to 50 % in all locations, namely 62.5 % for VC (2021), 50 % for Ma (2021), 62.5 % for L (2021), 75 % for S (2021) and 87.5 % for Sa (2021) (Table 2, Fig. 3B). Once more, the misclassified samples were, in general, allocated to sampling locations sharing the lowest d^2 with the original locations (Table 2, Fig. 4). For instance, the samples from VC (2021) were allocated to the Ma (2021) or L (2021) (Table 2, Fig. 4).

The elements that contributed the most to the dissimilarity in multi-elemental composition among harvesting locations were not the same for 2020 and 2021 (Fig. 5). While in 2020, the dissimilarities among locations were mainly due to variations in levels of Mg and Zn, with other elements presenting minor contributions (i.e., Ca, Ni, Pb, Rb, Sr and Y), in 2021, the main contributions were more evenly distributed with Y, Zn, Ca, Fe and Rb being among the most important (Fig. 5).

In the test using a model built with temporally mismatched samples, the samples collected in 2021 were correctly allocated to their corresponding 2020 locations in 55 % of the cases (Table 3). The locations presented the following allocation success: VC (2021) (0 %; Table 3, Fig. 6A), Ma (2021) (25 %; Table 3, Fig. 6B), L (2021) (93.75 %; Table 3, Fig. 6C) and S (2021) (62.5 %; Table 3, Fig. 6D).

4. Discussion

4.1. Seafood safety

Sea urchin gonads accumulate elements present in the environment and hence they are considered either as highly nutritive, due to the high levels of essential elements (Archana and Babu, 2016), or a risk for human health, if they are harvested from polluted areas (Bielmyer et al., 2012). The variation of mean levels of As (0.99–2.65 mg•Kg^{-1}), Cu (0.49–1.07 mg•Kg^{-1}), Pb (0–2.59 mg•Kg^{-1}) and Zn (40–142 mg•Kg^{-1}) measured in *P. lividus* gonads are, generally, in line with those previously

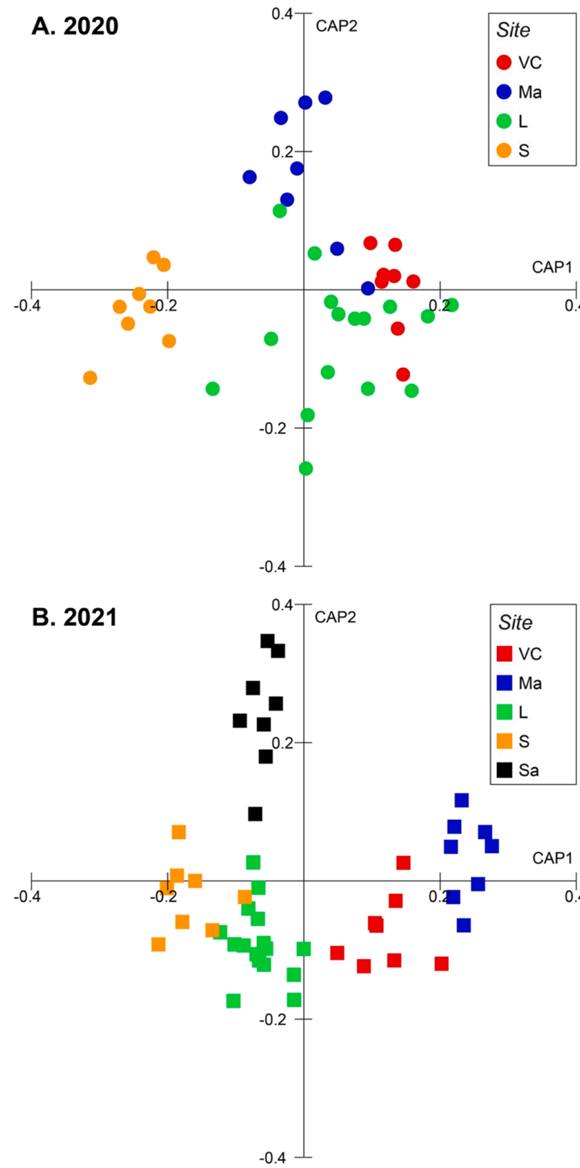

Fig. 3. Canonical Analysis of Principal (CAP) coordinates based on the elemental fingerprints of the *Paracentrotus lividus* gonads collected in four locations in 2020 and five locations in 2021 along the Portuguese Atlantic coast. Viana do Castelo - VC; Matosinhos - Ma; Lisbon - L; Sines - S; Sagres – Sa.

Table 2
Classification accuracy (by sampling location) of the Canonical Analysis of Principal (CAP) coordinates based on the elemental fingerprints of *P. lividus* gonads from four locations in 2020 and five locations in 2021 of the North Atlantic Portuguese coast. Viana do Castelo (VC), Matosinhos (Ma), Lisbon (L), Sines (S) and Sagres (Sa).

	Original Location	Classified Location					%Correct
		VC	**Ma**	**L**	**S**		
2020	**VC**	7	0	1	0		87.5
	Ma	2	6	0	0		75.0
	L	4	2	8	2		50.0
	S	0	0	0	8		100.0
	Total						**72.5**
2021		**VC**	**Ma**	**L**	**S**	**Sa**	
	VC	5	2	1	0	0	62.5
	Ma	4	4	0	0	0	50.0
	L	1	0	10	3	2	62.5
	S	0	0	2	6	0	75.0
	Sa	0	0	1	0	7	87.5
	Total						**66.7**

measured in the Northern Portuguese coast and other Atlantic and Mediterranean coasts (Camacho et al., 2018 and references therein), with the exception of the maximum levels of Pb and Zn, mainly from samples from S (2020). Nonetheless, in this study, *P. lividus* gonads from the Portuguese coast presented mean levels of potentially toxic elements (i.e., As, Pb and Zn) above the set thresholds, in at least one of the sampling locations. It is important to highlight, however, that the established levels used as a comparison in the present study were developed for mollusks, due to the absence of human consumption thresholds for sea urchin gonads. Mollusks are far more commonly consumed (independently of the group: cephalopods, bivalves or gastropods) than sea urchin gonads (FAO, 2021), an important factor in the calculation of the maximum levels recommended (FAO/WHO, 1995). As the levels of such elements in *P. lividus* gonads are not especially high when compared to other marine organisms, including mollusk cephalopods (Bonsignore et al., 2018), the levels presented by the *P. lividus* gonads above the established thresholds must be related to high environmental concentrations of these elements in the respective locations,

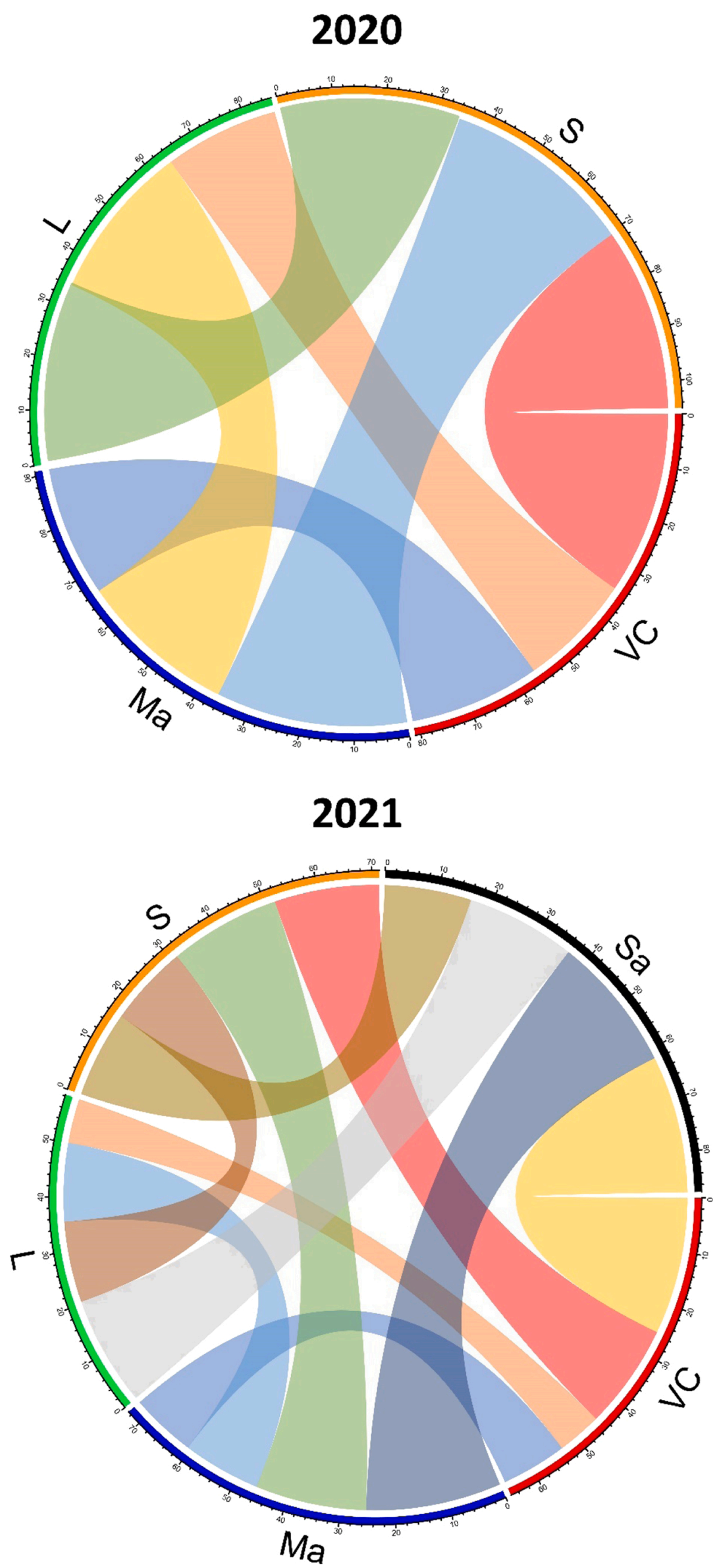

Fig. 4. Average square Euclidean distance (d^2) chord diagrams between sampling locations within each year (2020 and 2021) based on the elemental fingerprints of the *Paracentrotus lividus* gonads. Wider alluvials represent higher square Euclidean distances between paired sampling locations. Viana do Castelo - VC; Matosinhos - Ma; Lisbon - L; Sines - S; Sagres – Sa.

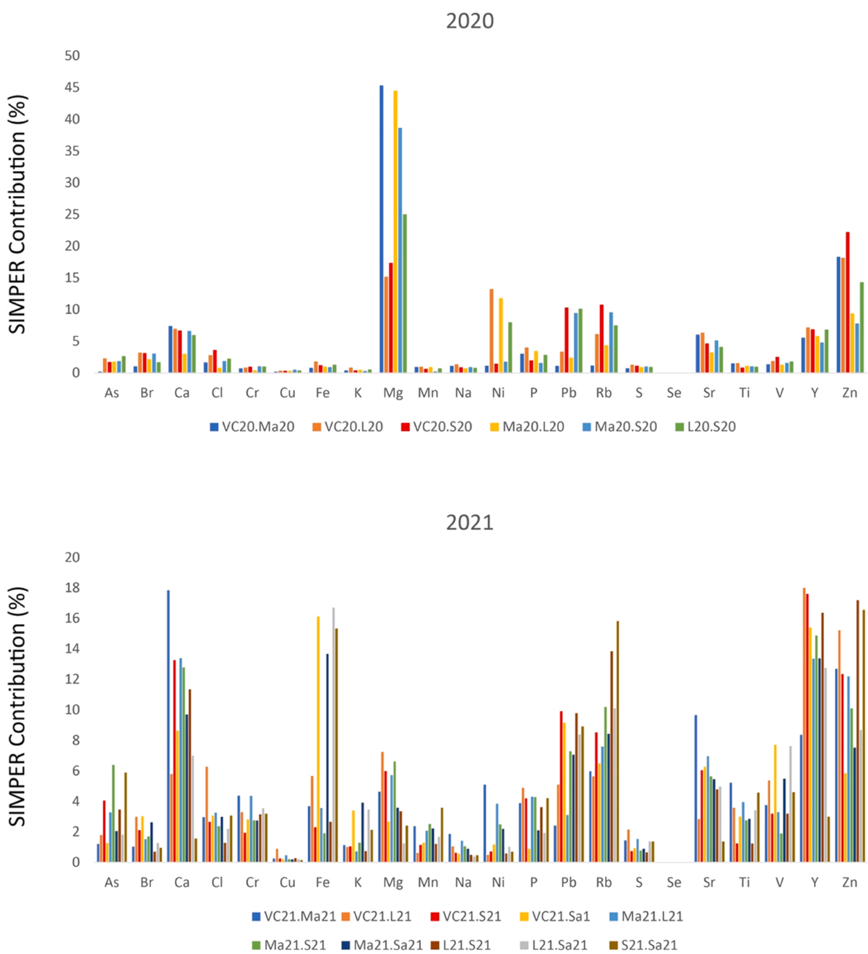

Fig. 5. Similarity percentages (SIMPER) based on Euclidean distances among the elemental fingerprints of the *Paracentrotus lividus* gonads collected in four locations in 2020 and five locations in 2021 along the Portuguese Atlantic coast. VC20.Ma20 – Viana do Castelo 2020 vs. Matosinhos 2020; VC20.L20 – Viana do Castelo 2020 vs. Lisbon 2020; VC20.S20 – Viana do Castelo 2020 vs. Sines 2020; Ma20.L20 – Matosinhos 2020 vs. Lisbon 2020; Ma20.S20 – Matosinhos 2020 vs. Sines 2020; L20.S20 – Lisbon 2020 vs. Sines 2020; VC21.Ma21 – Viana do Castelo 2021 vs. Matosinhos 2021; VC21.L21 – Viana do Castelo 2021 vs. Lisbon 2021; VC21.S21 – Viana do Castelo 2021 vs. Sines 2021; VC21.Sa21 – Viana do Castelo 2021 vs. Sagres 2021; Ma21.L21 – Matosinhos 2021 vs. Lisbon 2021; Ma21.S21 – Matosinhos 2021 vs. Sines 2021; Ma21.Sa21 – Matosinhos 2021 vs. Sagres 2021; L21.S21 – Lisbon 2021 vs. Sines 2021; L21.Sa21 – Lisbon 2021 vs. Sagres 2021; S21.Sa201 – Sines 2021 vs. Sagres 2021.

Table 3
Pinpoint of samples collected in 2021 in four locations along the Portuguese Atlantic coast using the model built with Canonical Analysis of Principal (CAP) coordinates based on the elemental fingerprints of *P. lividus* gonads collected in 2020. Viana do Castelo (VC), Matosinhos (Ma), Lisbon (L) and Sines (S).

Original Location	Classified Location				%Correct
	VC 2020	**Ma 2020**	**L 2020**	**S 2020**	
VC 2021	0	0	8	0	0.0
Ma 2021	3	2	3	0	25.0
L 2021	0	1	15	0	93.8
S 2021	1	1	1	5	62.5
Total					**55.0**

and not with particular features of this tissue. Moreover, and as abovementioned, *P. lividus* gonads are mostly consumed as delicacies, and therefore the total intake per day per person is not expected to reach dangerous values as it would be of concern considering other seafood products (fish for example), that are consumed as the main animal protein on a regular basis.

The input of trace elements into the environment can be through natural or anthropogenic sources (Richir and Gobert, 2016). Therefore, the high levels of As measured in *P. lividus* gonads from Ma can be either related to the proximity to a large fishing/cargo port (Port of Leixões), which is generally associated with high levels of environmental As (Mamindy-Pajany et al., 2013; OECD, 2010); or to the high geochemical baseline concentrations of As in the northern areas of mainland Portugal, embracing the basin of Douro River that reaches the ocean near Ma (Inácio et al., 2008). Nevertheless, the presence of levels of As above the set threshold in most samples, suggests that the baseline concentration of this element in Portuguese coastal waters is high. Regarding the high levels of Pb and Zn registered in the gonads from S (2020), these likely reflecting high environmental concentrations in this sampling location, like those found in nearby areas in coastal waters (Santos-Echeandía et al., 2012) and in sediments of a riverside where the effluents of a refinery and petrochemical industry were discharged (Nunes, 2015). The decrease in levels of Pb and Zn between years in samples from S can be related to interannual variation and in this particular case to reduced anthropogenic input, for example the closure in January 2021 of a Thermoelectric Facility, one of the major industries nearby Sines (Dimovska et al., 2014). Concomitant with an anthropogenic origin of these elements, however more studies are needed to prove this matter. Finally, the levels of Cu present in *P. lividus* gonads are not in line with high concentrations previously found in waters near the sampling locations (Santos-Echeandía et al., 2012), which suggests a physiologically regulation of this element to prevent the accumulation up to toxic levels (Powell et al., 2010).

4.2. Traceability

Knowing seafood provenance is nowadays a major concern to consumers, either regarding conservation of natural stocks to support the authorities in the regulation of harvesting, or food safety due to environmental contamination of harvesting locations (Gopi et al., 2019a). Despite these concerns, the high demand for sea urchin gonads led to the depletion of natural stocks in some locations (Andrew et al., 2002), which can result in harvesting restrictions (e.g., spatial limitations and temporal closures). Therefore, the development of efficient traceability tools to either inform the publictowards a more sustainable

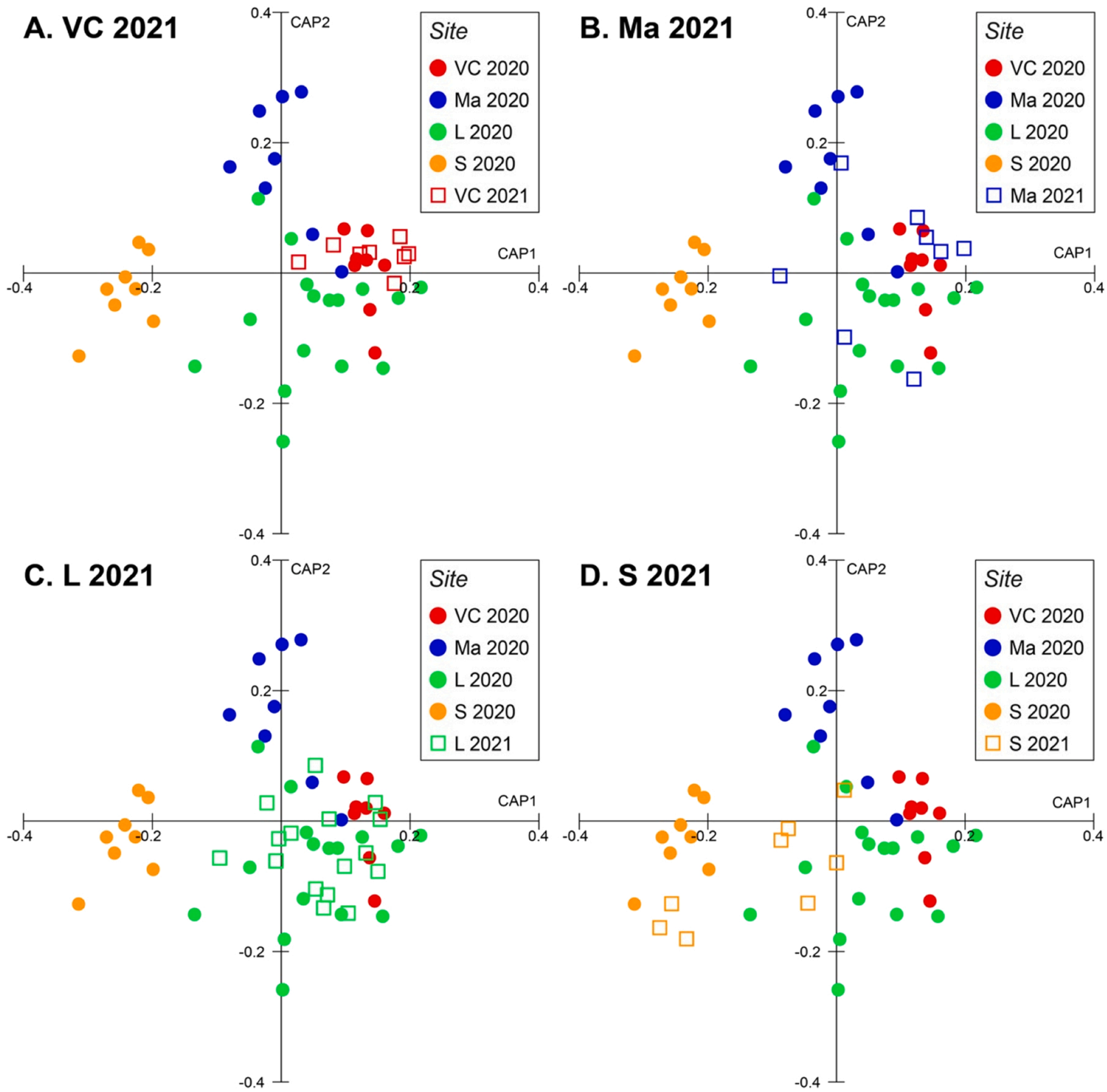

Fig. 6. Pinpoint of samples collected in 2021 in four sites along the Portuguese Atlantic coast using the model built with Canonical Analysis of Principal (CAP) coordinates, based on the elemental fingerprints of *Paracentrotus lividus* gonads collected in 2020. Viana do Castelo - VC; Matosinhos - Ma; Lisbon - L; Sines - S.

consumption, or to help marine authorities in the enforcement of regulations regarding the harvesting in some locations is paramount. To our knowledge, this is the first study evaluating the potential of the elemental fingerprint of *P. lividus* gonads (or any sea urchin species) to be used as a tracer of their geographic origin. As such, the present results were compared to geographic traceability studies focusing on edible tissues of other marine species. Despite the relatively high classification success obtained in this study (72.5 % for 2020; 66.67 % for 2021), they are below those obtained in traceability studies based on elemental fingerprints of echinoderms, namely the sea cucumber *Apostichopus japonicus* (88.2 %–94.1 %, Kang et al., 2018; 100 % Liu et al., 2012), as well as other species with low mobility, more precisely the sessile species king scallops *Pecten maximus* (82.7 %, Morrison et al., 2019), mussels *Mytilus galloprovincialis* (75–100 %, Costas-Rodríguez et al., 2010) and stalked barnacle *Pollicipes pollicipes* (70.0–84.4 %, Duarte et al., 2022c). Regardless of the phylogenetic and ecological difference, these results are more in line with those using the elemental fingerprints of fish muscle to trace their geographic origins, such as thornback ray *Raja clavata* (65.1 %, Duarte et al., 2022a), Asian seabass *Lates calcarifer* (72 %, Gopi et al., 2019b), or European seabass *Dicentrarchus labrax* (56–79 %, Farabegoli et al., 2018). Despite the promising results presented in this study, the optimization of traceability tools should always be pursued. Therefore, future sea urchin traceability studies should focus on, the combined analysis of tissues (Bennion et al., 2019) or biochemical signatures (e.g., stable isotopes) (Gopi et al., 2019b, 2019a; Ortea and Gallardo, 2015).

In each year, different elements were important for the discrimination of the harvesting locations, which suggest an annual variation in the chemical composition of the Portuguese coastal waters. Such temporal (seasonal and annual) variations reflected in the elemental fingerprints of marine organisms (Bennion et al., 2021; Morrison et al., 2019) can impair the traceability of the geographic origin of seafood if samples from different locations are not collected within a short time period. Despite the variation between the elemental profiles of *P. lividus* gonads harvested in 2020 and 2021, using the model based on samples collected in 2020, the samples collected in 2021 were correctly classified in 55 % of the cases, showing the potential to trace the provenance of sea urchins using models developed with temporally mismatched samples. The correct allocation was higher in the southern locations (L and S with respectively 97.5 % and 62.5 %), indicating that this approach is more promising in these locations, among which, S presented high levels of potentially toxic elements (i.e., Pb and Zn). Therefore, despite the high intra-annual variation in gonad index of sea urchins (de la Uz et al., 2018), and presumably of their elemental fingerprint (Guendouzi et al., 2017; Ouchene et al., 2021), the annual cycle of this index permitted the traceability, to some extent, of samples from these locations using a model based on samples collected in the previous year. In fact, the influence of the gonad index and the associated metabolic requirements, that differ according to season, on the elemental concentration in *P. lividus* gonads is still a matter for debate, with some authors describing positively correlations between them (Ouchene et al., 2021), while others concluding that they are negatively correlated (Guendouzi et al., 2017). Notwithstanding, part of intra-site variability observed in the sea urchin gonads elemental signatures, can be linked to the different reproductive stages of the individuals, given that collection was performed in two different months (November and July, in 2020 and 2021 respectively). Seasonal changes of gonad maturation stages of *P. lividus* have been described for the Portuguese coast, in which the majority of the individuals have partially or totally spawned in the summer period, whilst during fall the gonads are usually in recovery stages (Rocha et al., 2019).

5. Conclusions

The present study revealed that *P. lividus* gonads from some locations of the Portuguese coast.

presented levels above those recommended (for mollusks) for As, Pb and Zn. Furthermore, this study has unraveled the potential to use the elemental fingerprints of sea urchin gonads to trace their geographic origin, as well as to trace the harvesting location of *P. lividus* using models developed with samples from previous years. Therefore, the traceability tools developed in the present study can promote the decrease of potential food-safety risks associated with the trade of sea urchin gonads originated from polluted locations, as well as contribute to the maintenance of the natural stocks of this marine resource. Hereupon, future work should focus on: i) development of recommended levels of potentially toxic elements for sea urchin gonads, accounting with their features (i.e., average consumption, baseline levels of elements in gonads); ii) development of traceability tools based on the elemental fingerprints of other sea urchin tissues, such as spines, as they present different features (e.g., grows continuously) and applications (e.g., easiest to sample); iii) optimization of these traceability tools through the combination with different tissues (e.g., spines) or biochemical signatures (e.g., fatty acids or stable isotopes), as well as through further evaluation of the temporal variation (monthly, seasonal and annual) of elemental fingerprints of *P. lividus* gonads and its influence in the applicability of traceability tools.

CRediT authorship contribution statement

Renato Mamede: Conceptualization, Writing – original draft, Formal analysis, Investigation. **Irina A. Duarte**: Investigation, Writing – review & editing. **Isabel Caçador:** Writing – review & editing. **Susanne E. Tanner:** Investigation, Writing – review & editing. **Marisa Silva:** Resources, Investigation, Writing – review & editing, Project administration, Funding acquisition. **David Jacinto:** Resources, Investigation, Writing – review & editing. **Vanessa F. Fonseca:** Conceptualization, Writing – review & editing, Formal analysis, Investigation, Project administration, Funding acquisition. **Bernardo Duarte:** Conceptualization, Investigation, Project administration, Funding acquisition.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104764.

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