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Assessing the elemental fingerprints of cockle shells (*Cerastoderma edule*) to confirm their geographic origin from regional to international spatial scales

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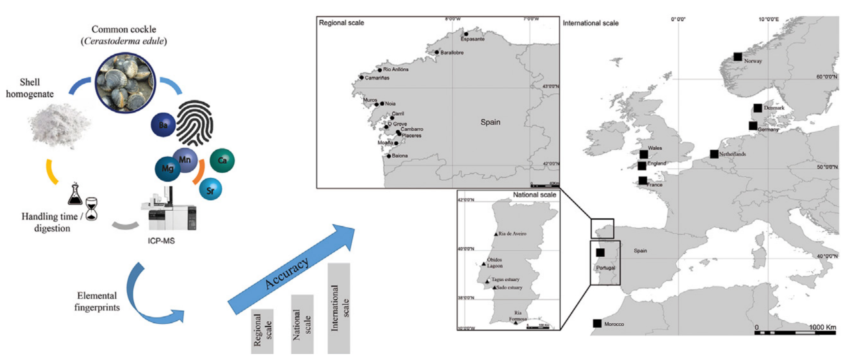
HIGHLIGHTS

- Elemental fingerprints (EF) of shells were determined for *Cerastoderma edule.*
- Cockles geographic origin was traced using a small homogenate portion of the valve.
- Cockles were traced within a regional, national and international spatial scale.
- Shifts in EF reliably discriminated cockles from different locations.
- Success rate of discrimination is positively correlated with spatial scale.

GRAPHICAL ABSTRACT



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ABSTRACT

Geographic origin is directly linked to the quality and commercial value of bivalves. The globalization of the seafood trade and the increasing number of fraudulent practices in the bivalves industry has prompted consumers to become increasingly aware on the geographic origin of the seafood they consume. To enhance consumers' confidence and allow authorities to effectively enforce regulations and contain risks that threaten public health, fast and accurate tools must be made available to confirm claims along the trade chain on the geographic origin of bivalves. In the present study the efficiency of using the elemental fingerprints of a small-homogenized subsample of the shell of common cockles (*Cerastoderma edule*) to confirm their harvesting location is evaluated at different spatial scales: i) regional (along the Galician coast (Spain) - Espasante, Barallobre, Rio Anllóns, Camariñas, Muros, Noia, Carril, Grove, Combarro, Placeres, Moaña, and Baiona), ii) national (along the Portuguese coast - Ria de Aveiro, Óbidos lagoon, Tagus estuary, Sado estuary and Ria Formosa), and iii) international (along the Northeast Atlantic coast - Hejeltefjorden (Norway), Nykobing Mors (Denmark), Sylt (Germany), Slikken van Viane (Netherlands), Roscoff (France), Plymouth (England), Swansea (Wales), Ria de Aveiro (Portugal) and Oualidia (Morocco). Results confirm that elemental fingerprints of bivalve shells are significantly different among locations and that they can be successfully used with high accuracy to discriminate the geographic origin of cockles at all spatial scales surveyed (97.2% at regional scale, 99.3% at national scale and 100% at international scale). Overall, elemental fingerprints of a

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small-homogenized subsample of the shell showed to be a replicable, low cost and fast tool to reliably trace the place of origin of cockles sampled at different spatial scales, with success rate of discrimination directly increasing with distance between collection sites.

1. Introduction

The global production of bivalve mollusks for human consumption has continuously grown over the last decades, mainly due to aquaculture production (FAO, 2020). From 2015 to 2018, the production of bivalves worldwide reached 60.34 million tons, representing up to 25% of the global production of aquaculture marine species (including seaweeds) (FAO, 2021). Globally, the major producers of bivalves include China (51 million tons), Republic of Korea (1.5 million tons) and Japan (1.4 million tons). Europe produced 2.4 million tons of bivalves, with major producers being Spain (972.3 thousand tons), France (532.0 thousand tons) and Italy (253.1 thousand tons) (FAO, 2020. However, the production of bivalves in Europe has been somehow stagnant due to spatial constraints, carrying capacity issues and disease outbreaks (Mente and Smaal, 2016; Smaal, 2002). To keep up with increasing demand, Europe has also increased the imports of bivalve mollusks (Hurtado-Bermúdez et al., 2019).

In an era of trade globalization and increasing complexity of trade chains, long pathways from harvesting to consumer invariably increase food safety risks associated with bivalve products (Leal et al., 2015; Oliveira et al., 2011). Bivalves are mainly filter-feeders, that feed on phytoplankton (e.g., diatoms, dinoflagellates) available in the water column. However, bivalves also accumulate metals, metalloids and algal toxins (Liu et al., 2017; Jiang et al., 2017), as well as microorganisms (e.g., *Salmonella* spp., *Vibrio* spp.) that may promote foodborne infections (Potasman et al., 2002). As a consequence of industry globalization, the chain of custody has become increasingly long, diffuse and less centralized (Yasuda and Bowen, 2006), making the traceability of bivalves' geographic origin imperative to safeguard food safety.

The European Union (EU) legislation (Regulation (EC) No 178/2002) (EC, 2002) requires that all stages of production, processing, and distribution of food or food-producing animals must be traced. In several markets, the self-confidence of consumers is compromised due to the mislabeling of seafood species, production method and geographic origin (Astill et al., 2019; Gopi et al., 2019; Jacquet and Pauly, 2008; Leal et al., 2015; Marko et al., 2004). Indeed, there is a growing concern from consumers to confirm the geographic origin of bivalves being purchased, thus trying to tell apart high-quality bivalves from poorer ones, which are often associated with illegal and/or fraudulent practices (Parrondo et al., 2021). Hence, to address current legal obligations and respond to the needs of a globalized seafood market, to enhance food safety, protect consumers and product brand reputation, it is important to develop efficient traceability tools associated with the production and trade of bivalves, encompassing both processing and trading pathways (Astill et al., 2019; Gopi et al., 2019; Leal et al., 2015).

Elemental fingerprints (EF), the geochemical profile recorded in hard biogenic structures (e.g., shells), have been successfully used to confirm the geographic origin/quantify population connectivity of multiple species from geographically close stocks, such as cockles (*Cerastoderma edule*; Ricardo et al., 2015; Ricardo et al., 2017), scallops (*Pecten maximus*; Morrison et al., 2019) and mussels (*Mytilus californianus* and *M. galloprovinciallis;* Becker et al., 2004, *M. edulis*; Bennion et al., 2019). These previous studies were focused on only one spatial scale, being able to determine the geographic origin of bivalves from <1 to 500 km apart using the whole shell. This ability to trace the place of geographic origin using EF of bivalve shells is possible since the shell material carries an imprint in its chemistry, crystal structure and morphology that mirrors the environment where specimens are actively precipitating shell carbonate (Steinhardt et al., 2016). This EF is also shaped by anthropogenic inputs (e.g., industrial, domestic, and agricultural activities) that contribute to unique elemental signatures in the shells of bivalves (e.g., Thébault et al., 2009; Zhao et al., 2017). From a traceability standpoint, the EF analysis of the whole shell, or the combined analysis of the shell and bivalve soft tissues, can achieve a remarkably high level of correct allocation of harvesting location (90–100%) (Bennion et al., 2019; Morrison et al., 2019; Ricardo et al., 2015; Ricardo et al., 2017; Sorte et al., 2013). Nonetheless, the use of this approach but in a faster, reliable and environmentally safer way to achieve a successful level of classification for bivalves originating from different spatial scales still deserves further investigation. Future works must be able to safeguard that methods such as the one here described can be successfully made available to legal authorities monitoring the trade of seafood and having to produce expert proof for prosecution in a timely, cost-efficient and reliable way.

The present study aimed to evaluate differences in EF of a small-homogenized portion of cockle shells from specimens collected at different spatial scales (along the Galician coast (Spain; regional spatial scale), along the Portuguese coast (national spatial scale) and along the Northeast Atlantic coast (international spatial scale)) and evaluate the accuracy of allocation of their place of origin at each of these spatial scales.

2. Material and methods

2.1. Sample collection and preparation

Thirty specimens of common cockle (*C. edule*, shell length > 30 mm and < 35 mm) were randomly collected in the intertidal zone from 29 important shellfish locations (approximately in a sampling area of 10,000 m^2 per location) by local fishermen. Sampling was performed at three different spatial scales comprising the natural geographic distributions of this species (from Norway to Morocco; Malham et al., 2012): at a regional spatial scale on twelve locations along the Galician coast (Spain) during the spring of 2018, namely at Espasante (Esp), Barallobre (Bar), Rio Anllóns (RAl), Camariñas (Cam), Muros (Mur), Noia (Noi), Carril (Car), Grove (Gro), Combarro (Com), Placeres (Pla), Moaña (Moa) and Baiona (Bai) (12 locations × 30 replicates = 360 samples) (Fig. 1a); at a national spatial scale on five location along the Portuguese coast during the summer of 2018, namely at Ria de Aveiro (RAv), Óbidos Lagoon (OL), Tagus estuary (TE), Sado estuary (SE) and Ria Formosa (RF) (5 location × 30 replicates = 150 samples) (Fig. 1b); and at an international spatial scale along the Northeast Atlantic coast during the summer of 2017, namely at Hejeltefjorden (Norway - NO), Nykobing Mors (Denmark - DK), Sylt (Germany - DE), Slikken van Viane (Netherlands - NL), Roscoff (France - FR), Plymouth (England - EN), Swansea (Wales - WAL), RAv (Portugal - PT) and Oualidia (Morocco - MO) (Fig. 1c). All samples were collected using hand-rakes, stored in sterile bags and kept refrigerated until being processed in the laboratory. Organic tissues were discarded and the right valve was washed with tap water and distilled water to remove mud and any debris, air-dried and stored for further analysis. If the right valve was damaged, the "left" valve was used (Ricardo et al., 2020).

2.2. ICP-MS

Prior to elemental analysis, all valves were prepared, and EF were determined using the method previously described in detail by Ricardo et al. (2020). Briefly, whole right valves were individually homogenized using a mortar grinder (RM 200, Retsch, Hann, Germany). Between the homogenization of samples, the mortar grinder was carefully cleaned with silicate followed by alcohol (70%) to avoid cross-contamination. Posteriorly, 0.2 g of homogenized valve was digested in high-purity concentrated (70% *w/v*) HNO_3 (Trace metals; Sigma-Aldrich) during 1 min and then diluted with Milli – Q (Millipore) water to a final acid concentration of 1–2% HNO_3. Total concentrations of aluminum (^{27}Al), barium (^{137}Ba), calcium

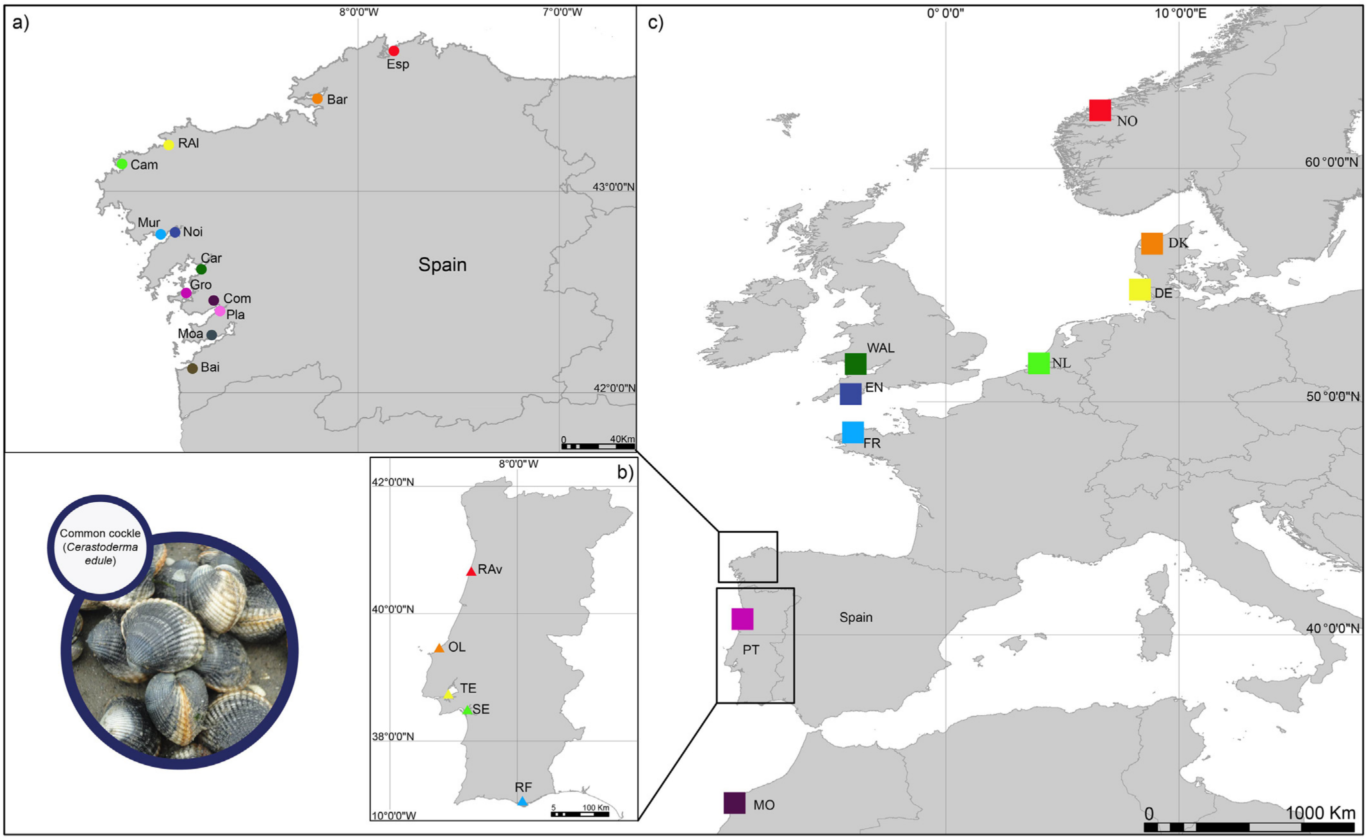

Fig. 1. Sampling locations of *Cerastoderma edule* at: a) a regional spatial scale (Galician coast; Spain): Espasante (Esp), Barallobre (Bar), Rio Anllóns (RAl), Camariñas (Cam), Muros (Mur), Noia (Noi), Carril (Car), Grove (Gro), Combarro (Com), Placeres (Pla), Moaña (Moa) and Baiona (Bai); b) a national spatial scale (mainland Portugal): Ria de Aveiro (RAv), Óbidos lagoon (OL), Tagus estuary (TE), Sado estuary (SE) and Ria Formosa (RF); and c) an international spatial scale (*Northeast Atlantic coast*): Hejeltefjorden (Norway - NO), Nykobing Mors (Denmark - DK), Sylt (Germany - DE), Slikken van Viane (Netherlands - NL), Roscoff (France - FR), Plymouth (England - EN), Swansea (Wales - WAL), Ria de Aveiro (Portugal - PT) and Oualidia (Morocco – MO). Coordinates are available on supplementary data (Table S1).

(^{44}Ca), cerium (^{140}Ce), cobalt (^{59}Co), iron (^{56}Fe), potassium (^{39}K), lanthanum (^{139}La), magnesium (^{24}Mg), manganese (^{55}Mn), sodium (^{243}Na), nickel (^{60}Ni), phosphorus (^{31}P), strontium (^{88}Sr) and yttrium (^{89}Y) were analysed using an Agilent 7700 ICP-MS equipped with an octopole reaction system (ORS) collision/reaction cell technology to minimize spectral interferences using the operation conditions summarized in Table S2. Germanium (^{72}Ge), Rhodium (^{103}Rh) and Terbium (^{193}Tb) were used as internal standards. For quality assurance and control (QA/QC), certified reference materials BCS-CRM-513 (SGT Limestone 1) were used. Mean recoveries for the selected elements in the SRM ranged from 93 to 126%, and the relative standard deviations (RSDs) for all replicates were < 10%.

2.3. Statistical analysis

Prior to perform any statistical analyses, the concentration of chemical elements present in cockle shells were converted to element/Ca ratios (mmol/mol) (Ricardo et al., 2015; Ricardo et al., 2017). The vegan adonis() function for permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was used to test for significant differences in EF of cockle shells among sampling locations. An analysis of variance (ANOVA) was then applied to test differences among locations for each individual elemental ratio.

The Boruta algorithm was applied to select the most important elemental ratios that may explain potential differences among the different locations sampled (Kursa et al., 2010). For a better understanding of our results, it is important to clarify that only the 5 elements that contributed the most for the characterization of the different locations within the three spatial scales were considered (Figs. S1–S3). A Random Forest model was used, considering the leave-one-out cross validation procedure, to evaluate the reliability of using EF of cockle shells to infer their geographic origin and a t-distributed stochastic neighbor embedding (t-SNE) was performed for data visualization based on selected features (van der Maaten and Hinton, 2008). All data were scale() transformed and used to produce a matrix using Euclidean distances with the vegdist() function in the vegan package in R (Oksanen et al., 2012). The Shapiro.test() function was used to test for normality, and the bartlett.test() function was used to test the assumption of homogenous variances. All statistical analyses were performed using R (R Development Core Team A, 2015).

3. Results

3.1. Regional spatial scale (Galician coast, Spain)

The EF of *C. edule* shells from twelve different locations along the Galician coast is shown in Fig. S4 (supplementary data). PERMANOVA revealed the existence of significant differences among the EF of cockle shells from different locations ($F = 49.7$, $R2 = 0.616$, $p = 0.001$). The top 5 elements selected by the Boruta algorithm included Co, Y, Ce, Fe and Mn (Fig. S1). The highest Co/Ca was registered in specimens from Car and Pla, being significantly different from specimens originating from other locations ($p < 0.05$), but not between these two ($p \geq 0.05$) (Fig. S5b). Specimens from Esp also registered the highest Fe/Ca and Mn/Ca, significantly differing from other locations (Fig. S5b–c). Cockles from Bai presented significantly higher levels of Ce/Ca, whereas Gro and Moa registered significantly higher levels of Y/Ca comparatively to other locations ($p < 0.05$, Fig. S5a–e).

The Random Forest model revealed a high classification accuracy (97.2%) when allocating the geographic origin of common cockles

Table 1
Classification success (by location at a regional scale) of Random Forest model for elemental fingerprints of *Cerastoderma edule* shells. Espasante (Esp), Barallobre (Bar), Rio Anllóns (RAl), Camariñas (Cam), Muros (Mur), Noia (Noi), Carril (Car), Grove (Gro), Combarro (Com), Placeres (Pla), Moaña (Moa) and Baiona (Bai).

	Predicted Location												Total per location	% correct (location)
	Esp	Bar	RAl	Com	Mur	Noi	Car	Gro	Cam	Pla	Moa	Bai		
Original Location														
Esp	29	0	1	0	0	0	0	0	0	0	0	0	30	96.7
Bar	0	28	0	0	0	0	0	0	2	0	0	0	30	93.3
RAl	0	0	29	0	0	0	0	1	0	0	0	0	30	96.7
Cam	0	0	0	1	0	0	0	0	28	0	0	1	30	93.3
Mur	0	0	0	0	29	1	0	0	0	0	0	0	30	96.7
Noi	0	0	0	0	0	30	0	0	0	0	0	0	30	100
Car	0	0	0	0	0	0	30	0	0	0	0	0	30	100
Gro	0	0	1	0	0	0	0	29	0	0	0	0	30	96.7
Com	0	0	0	28	0	0	0	1	1	0	0	0	30	93.3
Pla	0	0	0	0	0	0	0	0	0	30	0	0	30	100
Moa	0	0	0	0	0	0	0	0	0	0	30	0	30	100
Bai	0	0	0	0	0	0	0	0	0	0	0	30	30	100
Average classification success														97.2

collected along the Galician coast (Table 1). Specimens from Noi, Car, Pla, Moa and Bai showed the highest percentage of correct classification (100%), whereas for those originating from Esp, RAl, Cam, Mur, Gro and Com, a single specimen was misclassified, resulting in 96.7% of correct allocation of geographic origin. The highest level of misclassification (7%) was recorded for *C. edule* collected in Bar (Table 1; Fig. 2).

3.2. National spatial scale (Portuguese coast)

The mean values and standard deviations of elemental concentrations in *C. edule* shells from specimens collected on the five locations along the Portuguese coast are summarized in Fig. S6. PERMANOVA revealed the existence of significant differences among the EF of *C. edule* from different

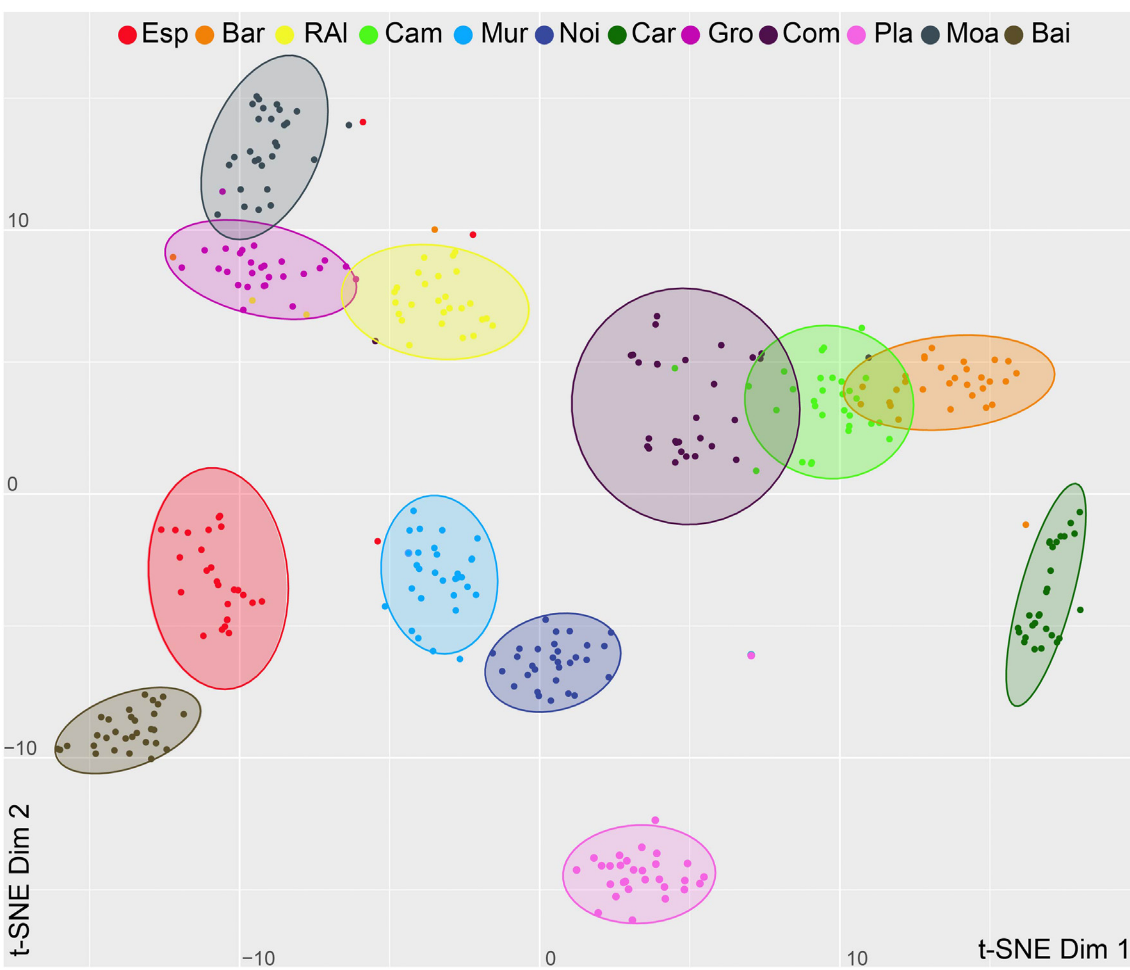

Fig. 2. The t-distributed stochastic neighbor embedding (t-SNE) feature visualization distribution based on elemental fingerprints of shells of *Cerastoderma edule* collected from twelve different locations along the Galician coast: Espasante (Esp), Barallobre (Bar), Rio Anllóns (RAl), Camariñas (Cam), Muros (Mur), Noia (Noi), Carril (Car), Grove (Gro), Combarro (Com), Placeres (Pla), Moaña (Moa) and Baiona (Bai).

Table 2
Classification success (by location at a national scale) of Random Forest model for elemental fingerprints of *Cerastoderma edule* shells. Ria de Aveiro (RAv), Óbidos lagoon (OL), Tagus estuary (TE), Sado stuary (SE) and Ria Formosa (RF).

	Predicted Location					Total per location	% correct (location)
	RAv	OL	TE	SE	RF		
Original Location							
RAv	30	0	0	0	0	30	100
OL	0	30	0	0	0	30	100
TE	0	0	29	1	0	30	96.7
SE	0	0	0	30	0	30	100
RF	0	0	0	0	30	30	100
Average classification success							99.3

locations ($F = 35.1$, $R2 = 0.492$, $p = 0.001$; Table S2). The Boruta algorithm revealed that the top 5 elemental ratios that contributed for the differences recorded among locations included Mn, Ba, Co, Fe and Ni (Fig. S2). Cockles from OL registered the lowest Co/Ca, Fe/Ca and Ni/Ca, showing significant differences from specimens originating from all other locations ($p < 0.05$) (Fig. S7). Specimens from TE displayed significantly higher levels Mn/Ca than those from the other locations ($p < 0.05$; Fig. S7). The values of Ba/Ca, Co/Ca and Fe/Ca were higher in cockle shells from RAv, with significant differences being recorded when compared with those from specimens originating from other locations ($p < 0.05$; Fig. S7). The highest levels of Ni/Ca were registered in specimens from RAv and SE, that were significantly different from those of other locations, but not from each other (Fig. S7).

The Random Forest model of the EF variation in the data set is shown in Table 2. Samples were correctly allocated in 99.3% of the cases, with specimens collected in RAv, OL, SE and RF displaying the highest percentage of correct allocation (100%). For cockles originating from TE a single specimen was misclassified, resulting in 96.7% of correct classifications (Table 2; Fig. 3).

3.3. International spatial scale (Northeast Atlantic coast)

The elemental composition standardized to Ca varied extensively between cockle shells from different geographic origins along the Northeast Atlantic coast (Fig. S8). PERMANOVA revealed the existence of significant differences among the eleven locations surveyed at this spatial scale ($F = 73.6$, $R2 = 0.705$, $p = 0.001$; Table S3). By using the Boruta algorithm it was possible to select the following top 5 elemental ratios: Mn, Ni, Y, Co and Fe (Fig. S3). Specimens from DE and MO were significantly different from those of other locations due to their higher concentrations of Y/Ca (Fig. S9). The values of Co/Ca were significantly higher ($p < 0.05$; Fig. S9) in cockle shells from PT comparatively with the rest of locations surveyed, whereas K/Ca, Mn/Ca and Ni/Ca were significantly higher in specimens from DK ($p < 0.05$; Fig. S9). The Random Forest model of elemental composition allowed to achieve an overall accuracy of allocation of 100% (Table 3; Fig. 4).

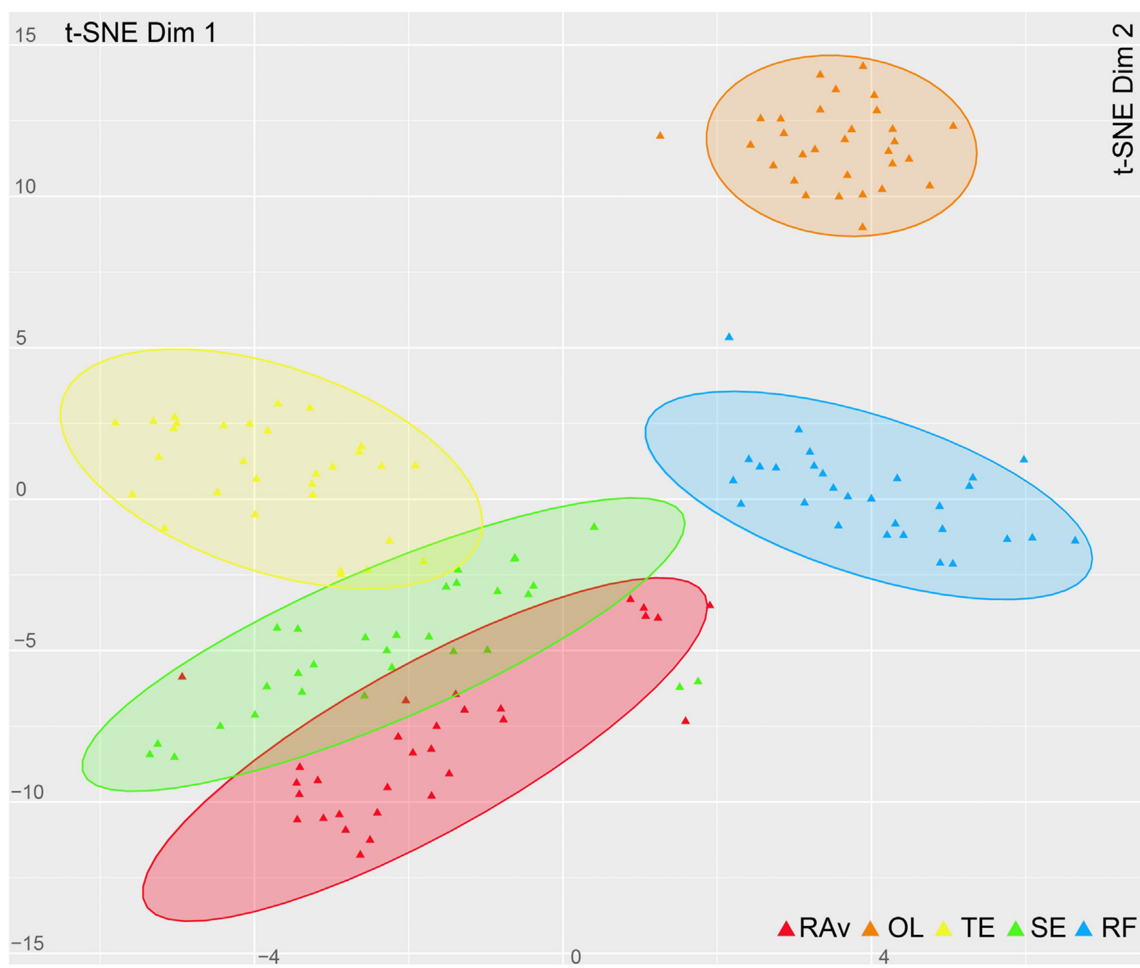

Fig. 3. The t-distributed stochastic neighbor embedding (t-SNE) feature visualization distribution based on elemental fingerprints of shells of *Cerastoderma edule* collected from five different locations along the Portuguese coast: Ria de Aveiro (RAv), Óbidos lagoon (OL), Tagus estuary (TE), Sado estuary (SE) and Ria Formosa (RF).

4. Discussion

4.1. Variability in elemental fingerprints at a regional spatial scale

The EF of cockle shells collected from different locations along the Galician coast displayed significant statistical differences. The Random Forest results showed an average success of 97.2% when identifyng the origin of cockles collected along the Galician coast (Table 1). These results are better than those reported for *M. edulis* in Ireland (regional spatial scale), where a discrimination success of 90% was achieved using the clean shell (Bennion et al., 2019). However, in this last study, higher discrimination rates were obtained through the combination of the EF of the shell and tissues (i.e. foot and periostracum). Specimens collected in Pla and Car registered the highest levels of Co/Ca when compared to cockles sampled in all other locations along the Galician coast. The concentration of Co in these locations could be associated with anthropogenic impacts (industry, aquaculture, port activities, fishing, tourism) (Prego and Cobelo-Garcia, 2003). Concerning the higher values of Fe/Ca and Mn/Ca, these are likely related to geogenic sources (Planquette et al., 2011; Röllin and Nogueira, 2019). It is, therefore, possible that ultrabasic rocks occurring in Ría of Ortigueira may have contributed to the abundance of these elements in Esp (Otero et al., 2000). The highest ratios of two rare earth elements detected (Ce/Ca and Y/Ca) in cockle shells originating from Bai and Moa (respectively) are likely associated with the geochemical characteristics of Ría de Vigo sediments. Indeed, these are mainly composed of igneous (alkaline and calc-alkaline granites) and metamorphic rocks (mainly mica schist and gneiss) that are associated to a large variety of minerals with rare earth elements as principal sources (Flemming and Bartoloma, 2009).

4.2. Variability in elemental fingerprints at a national spatial scale

Cockle shells along the Portuguese coast also displayed significantly different EF. The Random Forest analyses showed a discrimination rate of 99.7%, with this value being higher than that reported by Ricardo et al., 2017 for the common cockle *C. edule* in the same area (90%) but using the EF of the whole shell. The EF displayed by cockles from OL revealed the lowest levels of Co/Ca, Fe/Ca and Ni/Ca when compared to specimens sampled in all other locations along the Portuguese coast. This finding suggests that common cockles in OL may be less exposed to these pollutants, likely a consequence of the lower anthropogenic pressure over the surrounding lands of this coastal lagoon (Carvalho et al., 2006; Cortesão and Vale, 1995; Monterroso et al., 2003; Vale, 1990). High concentrations of metals, in particular Mn, can be associated with multiple anthropogenic sources (e.g., industrial, domestic, and agricultural activities) (Bernárdez et al., 2012). The presence of high levels of Mn/Ca in cockles from TE had already been previously reported by Ricardo et al. (2017) and its environmental input could be associated with historical anthropogenic impacts (namely metal industries) (Vale et al., 2008). The dissolved form of Mn (i.e. Mn^{2+}) occurs under reducing conditions (Schulz and Zabel, 2006) and a study carried out by Schöne et al. (2021) showed an inverse relationship between low oxygen levels and high levels of Mn/Ca in aragonitic bivalve shells, namely *Arctica islandica*. In estuarine environments, sediments are naturally characterized by anoxic conditions, likely explaining the high incorporation of this element in cockle shells.

The higher ratio of Ba/Ca recorded in cockle shells from specimens surveyed in RAv is in line with those already available in the literature for specimens collected in 2015 exactly in the same location (Ricardo et al., 2017). Freshwater inputs and nutrient runoffs from the fertile lands used for agricultural purposes in the margins of RAv can promote an increase in primary production and contribute to the occurrence of diatom blooms, thus increasing the bioavailability of Ba (Lopes et al., 2007; Thébault et al., 2009; Vander Putten et al., 2000). Anthropogenic pressures, such as agriculture and domestic effluents, promote the build-up of metals in surface water and sediment, likely being responsible for the significantly higher levels of Co/Ca, Fe/Ca and Ni/Ca recorded in cockle shells from RAv and Se (Caeiro et al., 2017; Mil-Homens et al., 2014).

Table 3
Classification success (by location at an international scale) of Random Forest model for elemental fingerprints of *Cerastoderma edule* shells. Hejeltefjorden (Norway - NO), Nykobing Mors (Denmark - DK), Sylt (Germany - DE), Slikken van Viane (Netherlands - NL), Roscoff (France - FR), Plymouth (England - EN), Swansea (Wales - WAL), Ria de Aveiro (Portugal - PT) and Oualidia (Morocco – MO).

Original Location	Predicted Location									Total per location	% correct (location)
	NO	DK	DE	NL	FR	EN	WAL	PT	MO		
NO	30	0	0	0	0	0	0	0	0	30	100
DK	0	30	0	0	0	0	0	0	0	30	100
DE	0	0	30	0	0	0	0	0	0	30	100
NL	0	0	0	30	0	0	0	0	0	30	100
FR	0	0	0	0	30	0	0	0	0	30	100
EN	0	0	0	0	0	30	0	0	0	30	100
WAL	0	0	0	0	0	0	30	0	0	30	100
PT	0	0	0	0	0	0	0	30	0	30	100
MO	0	0	0	0	0	0	0	0	30	30	100
Average classification success											100

4.3. Variability in elemental fingerprints at an international spatial scale

The significant differences registered in the EF of cockle shells collected along the Northeast Atlantic coast revealed that this approach can be used to trace geographic origin with high level of certainty (100%, Table 3). Results reported at international spatial scale to successfully allocate sampled specimens to their geographic origin are higher than those reported in previous studies addressing other bivalves (e.g., *M. edulis*) along the coast of the Gulf of Maine (68%) (Sorte et al., 2013). Common cockles collected from MO displayed the highest levels of Y/Ca, while highest levels of La/Ca were registered in cockle shells from NO and MO when compared to all other locations surveyed along the Northeast Atlantic coast. Till et al. (2017) analysed in 2011 surface samples obtained from cruise track North Atlantic GEOTRACES GA03 transect from Woods Hole, MA, to Praia, Cape Verde. The results showed high La concentrations on the western side of the transect (possibility of a fluvial end-member), and also high Y concentrations (consistent with a fluvial end-member). These findings, along with the levels of rare earth elements recorded in cockle shells, suggest that in NO and MO these bivalves may be exposed to waters from acidic geogenic sources (Banks et al., 1999; Mejjad et al., 2016). It is also worth highlighting that the availability of rare earth elements in the water is significantly affected by climate (Bao and Zhao, 2008). The sampling site at MO is located further south than other sampling locations along the Northeast Atlantic. Mejjad et al. (2016) concluded that the major sources of rare earth elements in the Oualidia lagoon sediments are due to the input of terrigenous material and that incorporation from seawater enriched with rare earth elements results from a prevailing coastal upwelling activity in the Atlantic Moroccan coast. The same authors also remark that the levels of rare earth elements in that location may also be due to anthropogenic activities in adjacent areas. Concentrations of Y in surface waters are lower in the North Atlantic when compared with the Mediterranean Sea, likely due to the different strengths of Y inputs to the ocean from fluvial and aeolian sources, although deep water concentrations of Y display a reverse trend (Alibo et al., 1999). The presence of Ni in cockles from DK had already been reported for *Mytilus edulis*. This could be associated with the presence of anthropogenic pressures, such as port activities and metal industries (Maar et al., 2018). The high levels of Mn present in seawater are likely associated with geogenic sources (Röllin and Nogueira, 2019). The EF of cockle shells originating from PT (in RAv) in 2017 revealed significantly higher Co/Ca. This finding was also registered in 2018, in the sampling performed at the national spatial scale. The potential explanation for the high levels of Co/Ca has been detailed above (see Section 4.2.).

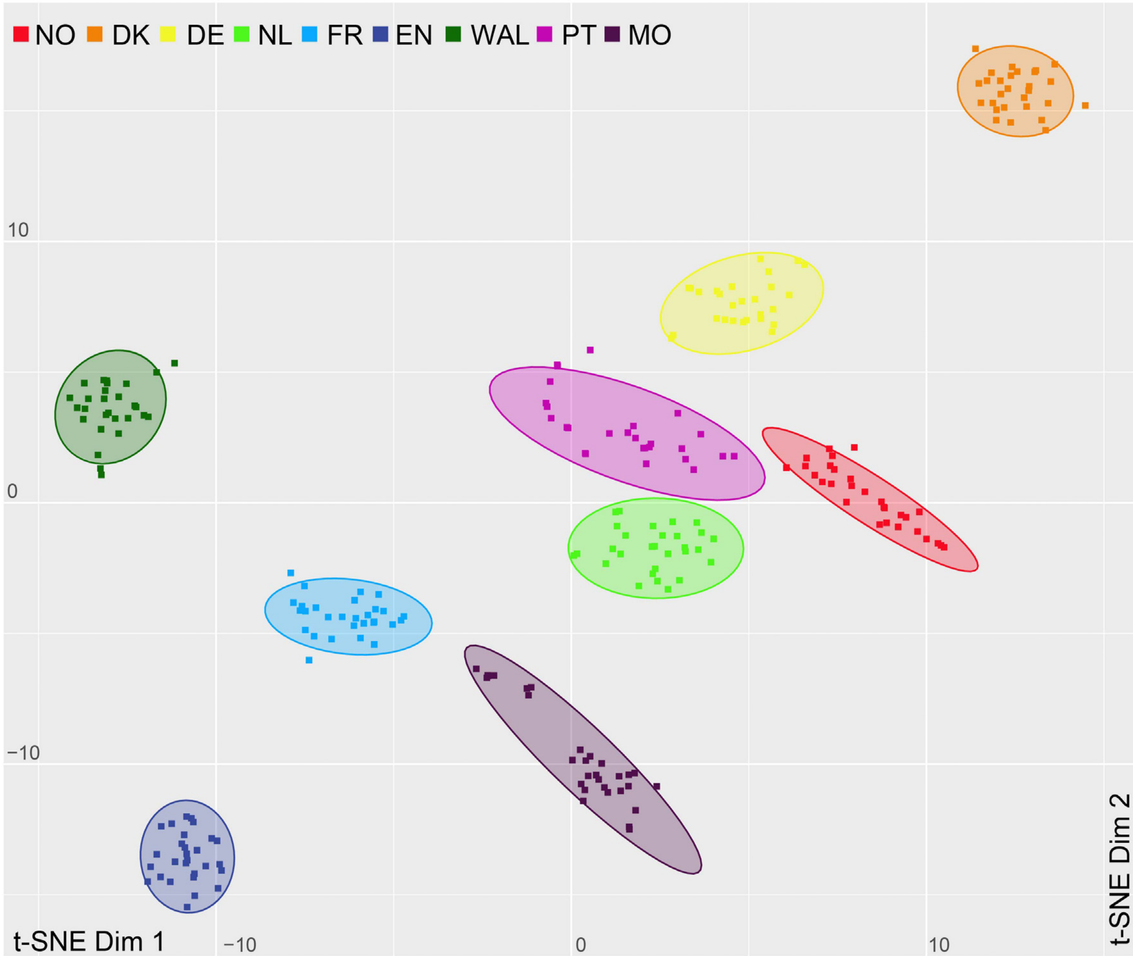

Fig. 4. The t-distributed stochastic neighbor embedding (t-SNE) feature visualization distribution based on elemental fingerprints of shells of *Cerastoderma edule* collected from twelve different locations along the Northeast Atlantic coast: Hejeltefjorden (Norway - NO), Nykobing Mors (Denmark - DK), Sylt (Germany - DE), Slikken van Viane (Netherlands - NL), Roscoff (France - FR), Plymouth (England - EN), Swansea (Wales - WAL), Ria de Aveiro (Portugal - PT) and Oualidia (Morocco – MO).

5. Conclusion

The present study reinforces the potential of using EF of a small-homogenized portion of bivalve shells to successfully discriminate their geographic origins at a regional, national and international spatial scale. This study provides further insights into a replicable, low cost and faster method to verify the geographic origin of bivalves. At present, EF are certainly one of the best available tools to verify claims on geographic origin and enforce the traceability of bivalves collected at different spatial scales. Future studies should extend this approach to other commercially important bivalve species and confirm the suitability of using this technique as a "one-size-fits-all" approach. The success rate of discrimination was shown to be positively correlated with the spatial scale being addressed. Therefore, it will also be important to continue to investigate the minimum spatial resolution at which this approach can still yield high success levels on the correct allocation of geographic origin, as well as its potential use in real case scenarios using independent data.

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Declaration of Competing Interest

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

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