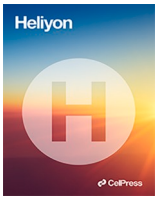
Heliyon 10 (2024) e25872

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Effects of H_2O_2 pretreatment on the elemental fingerprints of bivalve shells and their implications for the traceability of geographic origin

Renato Mamede [a,*], Carla Patinha [b], Patrícia Martins [a], Eduardo Ferreira da Silva [b], Ricardo Calado [a], Fernando Ricardo [a,**]

[a] *ECOMARE, CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Santiago University Campus, 3810-193, Aveiro, Portugal*
[b] *GEOBIOTEC, Department of Geosciences, University of Aveiro, Santiago University Campus, 3810-193, Aveiro, Portugal*

ARTICLE INFO

Keywords:
Seafood
ICP-MS
Biogeochemical tools
Methodological optimization
Hydrogen peroxide

ABSTRACT

The fraudulent mislabelling of seafood geographic origin has been growing due to complex supply chains and growing consumer demand. To address this issue, seafood traceability tools, such as those based on elemental fingerprints (EF) of bivalve shells, have been successfully used to confirm their harvesting location. However, despite the usefulness of these methodologies, there is still room for optimization. Therefore, this study evaluated the effects of a routine procedure during bivalve shells preparation for ICP-MS analysis – their pretreatment with H_2O_2 to remove organic components. More specifically, the present study evaluated the effects of H_2O_2 on i) the elemental fingerprints of shells of two bivalve species (*Ruditapes philippinarum* and *Cerastoderma edule*) from four different locations over the north-western and the western Iberian coast, and ii) their influence on the accuracy of models (based on the EF of shells) used to confirm the geographic origin of these species. Significant differences were observed between untreated and pretreated shells of *R. philippinarum* (p within location ranging from 0.0001 to 0.0011) and *C. edule* (p ranging from 0.0001 to 0.0007 for *C. edule*) for both their elemental fingerprints as a whole and several individual elements. The accuracy of the models employed to determine the origin of the two bivalve species, using i) untreated shells, ii) pretreated shells, and iii) both pretreated and untreated shells grouped per location, was high, with the models accurately predicting the geographic origin of 100, 90 and 95% of *R. philippinarum* and 95, 100 and 95% of *C. edule*, respectively. These results show that the shifts in the EF of bivalve shells promoted by treating them with H_2O_2 prior to ICP-MS analysis did not affect the accuracy of the models used to confirm the geographic origin of both bivalve species. Therefore, the need to pre-treat bivalve shells with H_2O_2 can be dismissed in future studies addressing the traceability of bivalves when using ICP-MS, thus contributing to reducing environmental impacts and economic costs associated with this procedure, as well as the time required to obtain results.

* Corresponding author.
** Corresponding author.
E-mail addresses: renatomamede@ua.pt (R. Mamede), fafr@ua.pt (F. Ricardo).

https://doi.org/10.1016/j.heliyon.2024.e25872
Received 28 August 2023; Received in revised form 2 February 2024; Accepted 5 February 2024
Available online 10 February 2024

1. Introduction

Global seafood production has been increasing consistently for the last decades, as a way to ensure food security and nutritional quality for a growing world population [1,2]. Seafood is generally considered to be highly nutritional in terms of minerals, vitamins, omega-3 fatty acids, and high-value proteins [2,3], and due to its economic value, highly complex food chains, and increasing consumer demand, the fraudulent mislabelling of its origin has been growing [4,5]. The mislabelling of seafood origin is generally associated with illegal, unreported, and unregulated fishing (IUU), which causes food safety issues if seafood originated from polluted locations; moreover, it also impairs the fair valuation of seafood from law-abiding producers, as they must compete with fraudulent producers, and makes the management of natural stocks by authorities a more challenging task due to uncontrolled harvesting in restricted areas [5–9]. At the environmental level, IUU fishing can have significant ecological impacts, posing a serious threat to the conservation of biodiversity and the proper functioning of natural ecosystems [10].

The public awareness of such problems elicited a call to action by authorities, which fostered the development of seafood traceability and authentication laws, guidelines, and practices [11–13]. The traceability of the geographic origin of seafood is based on the recognized influence that local environmental chemistry (i.e., resulting from the influence of seawater and sediments) and local physical conditions (e.g., temperature and pH) have on the biochemical composition of seafood tissues [14,15]. Therefore, diverse biogeochemical signatures of seafood (e.g., elemental, fatty acids, and stable isotopes) have been successfully applied to confirm its geographic origin [7,16–20]. Regarding elemental fingerprint analysis, several analytical techniques, including Inductively Coupled Plasma (ICP) methods (e.g., ICP-MS, ICP-OES, or ICP-AES) and X-ray fluorescence-based methods (e.g., TXRF, WDXRF, or EDXRF), have been employed to analyze these biogeochemical signatures in both soft and hard tissues of seafood [6,20,21]. Tools based on elemental fingerprints (EF) of bivalve shells have already been developed and validated for diverse species, such as common cockles (*Cerastoderma edule*, [18,22,23]), Manila clams (*Ruditapes philippinarum*, [24,25]), grooved carpet shell clams (*Ruditapes decussatus*, [25]), king scallops (*Pecten maximus*, [26]), blue mussels (Mytilus edulis, [27,28]), and Mediterranean mussels (*Mytilus galloprovincialis*, [29]).

The use of shells in the confirmation of the geographic origin of mollusks is particularly relevant because mollusk shells are metabolically inert, preserving a record of the chemical elements incorporated during growth and presenting no degradation after harvesting [30]. This contrasts with the less stable soft tissues which feature elemental turnover throughout development and require

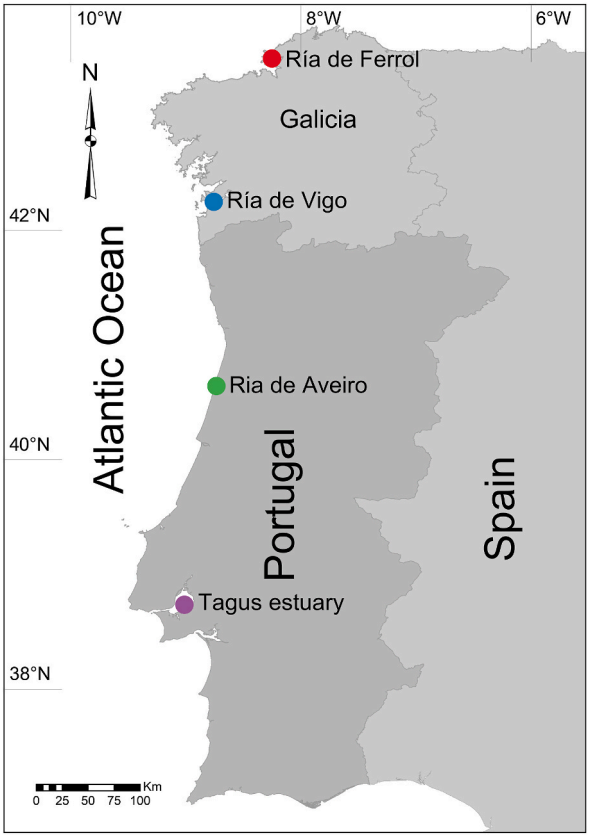


Fig. 1. Sampling locations of *Ruditapes philippinarum* and *Cerastoderma edule* along the NW and W Iberian coasts: Ría de Ferrrol (RFe: 43°27′48″N 8°11′23″O), Ría de Vigo (RV:42°12′00.20″N, 8°48′03.20″W), Ria de Aveiro (RAv:40°39′57.17″N, 8°43′24.70″W), and the Tagus Estuary (TE:38°44′5.18″N, 9°3′37.83″W). The map was created using ArcGIS v10.2.2.

more effort to avoid degradation after sampling [30,31]. Bivalve shells are mainly (95–99.9%) composed of calcium carbonate ($CaCO_3$), with the remaining portion consisting of an organic matrix [32,33]. When performing elemental analysis, it is customary to pretreat shells using one of several chemical reagents (acetone, H_2O_2 (hydrogen peroxide), NaOH (sodium hydroxide), or NaOCl (sodium hypochlorite)) or a combination thereof, despite the inherent risk to this procedure to partially remove $CaCO_3$ and the organic matrix of the shell, along with the elements therein [34,35]. This pretreatment is employed to prevent potential interference of elements present in the periostracum and any other foreign organic matter [22,34,36]. Traceability studies using the EF of bivalve shells, commonly use H_2O_2 for the pretreatment of shells and, despite the associated risks of leaching some elements [34,35], highly accurate predictive models have still been achieved [18,22,23,25,26,37].

Therefore, building upon previous studies that contributed to the optimization of seafood traceability tools as a way to provide faster results, reduce methodological costs, and decrease environmental impacts due to chemical residues [37,38], the present study aimed to evaluate the influence of using H_2O_2 on i) the EF of shells of two aragonitic bivalve species (*R. philippinarum* and *C. edule*), and ii) the accuracy of models used to determine the geographic origin of these species when using EF derived from either pretreated or untreated shells, as well as both pretreated and untreated shells together.

2. Material and methods

2.1. Study areas and sample collection

Five specimens of two bivalve species, *R. philippinarum* and *C. edule* ($\geq$40 mm for *R. philippinarum* and $\geq$25 mm for *C. edule*), were collected in the summer of 2018 (July and August) from four locations over the north-western and the western Iberian coast: Ría de Ferrol (RFe), Ría de Vigo (RV), Ria de Aveiro (RAv), and the Tagus estuary (TE) (4 locations $\times$ 2 species $\times$ 5 specimens = 40 samples, Fig. 1). These species were selected because they rank among the most economically relevant bivalves currently captured in the study area [39,40]. All samples were collected by hand-raking, stored in aseptic plastic bags, and refrigerated until arrival to the laboratory, where the specimens were cleaned of mud and debris with distilled water and taxonomically confirmed through recommended bibliography [41,42]. The valves were separated, and soft tissues were removed using ceramic-coated blades, plastic tip tweezers and stored for further analysis.

2.2. Sample preparation

Prior to elemental analysis, both the right and left valves of each specimen were prepared following the method described by Ricardo et al. [38], which reported that for traceability purposes, the EF of the right and left valves of bivalves can be used interchangeably. However, the right valves (from now termed as "pretreated shells") were fully soaked in high-purity H_2O_2 (30% w/v) (AnalaR NORMAPUR, VWR Scientific Products), overnight (14–16 h) to remove the periostracum and other organic matter [22], while the left valves (from now termed as "untreated shells") were not exposed to the treatment with H_2O_2. Each valve (pretreated and untreated) was then powdered in a mortar grinder (RM 200, Retsch, Hann, Germany), which was cleaned with silicate and alcohol between samples to avoid any cross-contamination. Approximately 0.2 g of powdered sample was digested in 1 mL of high-purity concentrated HNO_3 (70% w/v), with this solution being diluted with Milli – Q (Millipore) water to a final concentration of 1–2% HNO_3 [38].

2.3. ICP-MS analysis

Total concentrations of silver (Ag), aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), calcium (Ca), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), lead (Cu), dysprosium (Dy), erbium (Er), europium (Eu), iron (Fe), gadolinium (Gd), holmium (Ho), potassium (K), lanthanum (La), lutetium (Lu), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), neodymium (Nd), nickel (Ni), phosphorus (P), lead (Pb), praseodymium (Pr), rubidium (Rb), antimony (Sb), samarium (Sm), tin (Sn), strontium (Sr), thallium (Tl), thulium (Tm), uranium (U), vanadium (V), tungsten (W), ytterbium (Yb), yttrium (Y), and zinc (Zn) were analyzed using an Agilent 7700 ICP-MS equipped with an octopole reaction system (ORS) collision/reaction cell technology to minimize spectral interferences. The operating conditions are those summarized in supplementary material (Table S1). Germanium (Ge), Rhodium (Rh), and Terbium (Tb) were used as internal standards. For quality assurance and control (QA/QC) reagent blanks, analytical duplicates and BCS-CRM-513 (SGT Limestone 1) reference materials were also digested to determine the accuracy of the analytical and digestion procedures applied. Results of method blanks were always below the detection limit, while the mean recoveries for the selected elements ranged from 90 to 122%, and the relative standard deviations (RSDs) for all replicates being $<$10%.

2.4. Data and statistical analysis

For both bivalve species, the concentration of elements in their shells was expressed as a ratio to calcium (mg/mg) to minimize total mass effects [18,22,23,25]. A permutational analysis of variance (PERMANOVA) was performed to evaluate the existence of significant differences ($p < 0.05$) among locations and treatments. For both species, the standardized (i.e., subtraction of the mean (across all samples) and divide by the standard deviation of that variable) EF of the shells were compared in a two-way crossed model with two fixed factors: location, with four levels (RFe, RV, RAv and TE); and treatment, with two levels (pretreated and untreated). Moreover, to further investigate whether significant differences ($p < 0.05$) existed between the EF of pretreated and untreated shells, pair-wise

comparisons for factor treatment within the same location were performed. For each element, under original scaled values, nonparametric Kruskal–Wallis post hoc tests with Bonferroni correction were performed to investigate whether significant differences ($p < 0.05$) existed between pretreated and untreated shells from bivalves originating from the same location.

To investigate the influence of H_2O_2 on the accuracy of models used to trace the geographic origin of *R. philippinarum* and *C. edule*, three Random Forest [43,44] models were built for each species using: i) only untreated shells (4 locations x 5 specimens = 20 samples; Groups: untreated Ría de Ferrol (unRFe), untreated Ría de Vigo (unRV), untreated Ria de Aveiro (unRAv), and untreated Tagus estuary (unTE)); ii) only pretreated shells (4 locations x 5 specimens = 20 samples; Groups: pretreated Ría de Ferrol (prRFe), pretreated Ría de Vigo (prRV), pretreated Ria de Aveiro (prRAv), and pretreated Tagus estuary (prTE); iii) using all samples, with pretreated and

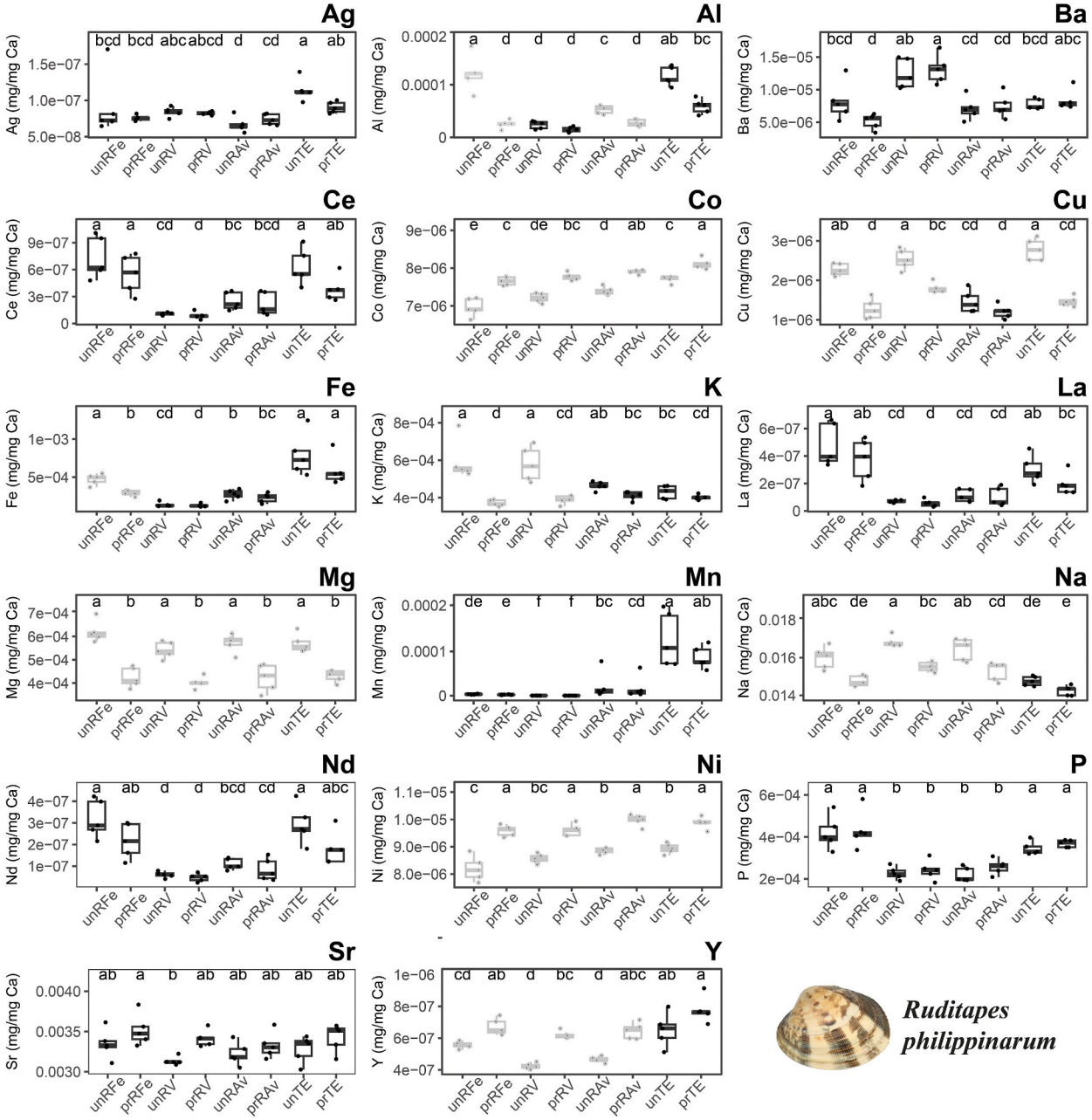


Fig. 2. Element-to-Ca ratios (mg/mg Ca) of *Ruditapes philippinarum* shells (pretreated and untreated) from four locations along the NW and W Iberian coast: untreated Ría de Ferrol (unRFe), pretreated Ría de Ferrol (prRFe), untreated Ría de Vigo (unRV), pretreated Ría de Vigo (prRV), untreated Ria de Aveiro (unRAv), pretreated Ria de Aveiro (prRAv), untreated Tagus estuary (unTE), and pretreated Tagus estuary (prTE). Different statistical letters (a, b, c, and d) denote significant differences between the sampling sites at $p < 0.05$. Significant differences ($p < 0.05$) between treatments (pretreated and untreated) within the same location are highlighted with grey boxplots.

untreated shells being grouped per location (4 locations x 10 specimens = 40 samples; Groups: Ría de Ferrol (RFe), Ría de Vigo (RV), Ria de Aveiro (RAv), and the Tagus estuary (TE)). The evaluation of model accuracy, which refers to the correct allocation of samples to their origin, was conducted using confusion matrices obtained through the application of leave-one-out cross-validation.

The PERMANOVA was performed using PRIMER v7 with the add-on PERMANOVA + [45,46], whereas the Kruskal–Wallis tests, boxplots, and random forest classifiers were performed in the R statistical environment (v. 4.1.3) [47] using the "agricolae", "ggplot2", and "randomForest" packages, respectively [44,48,49].

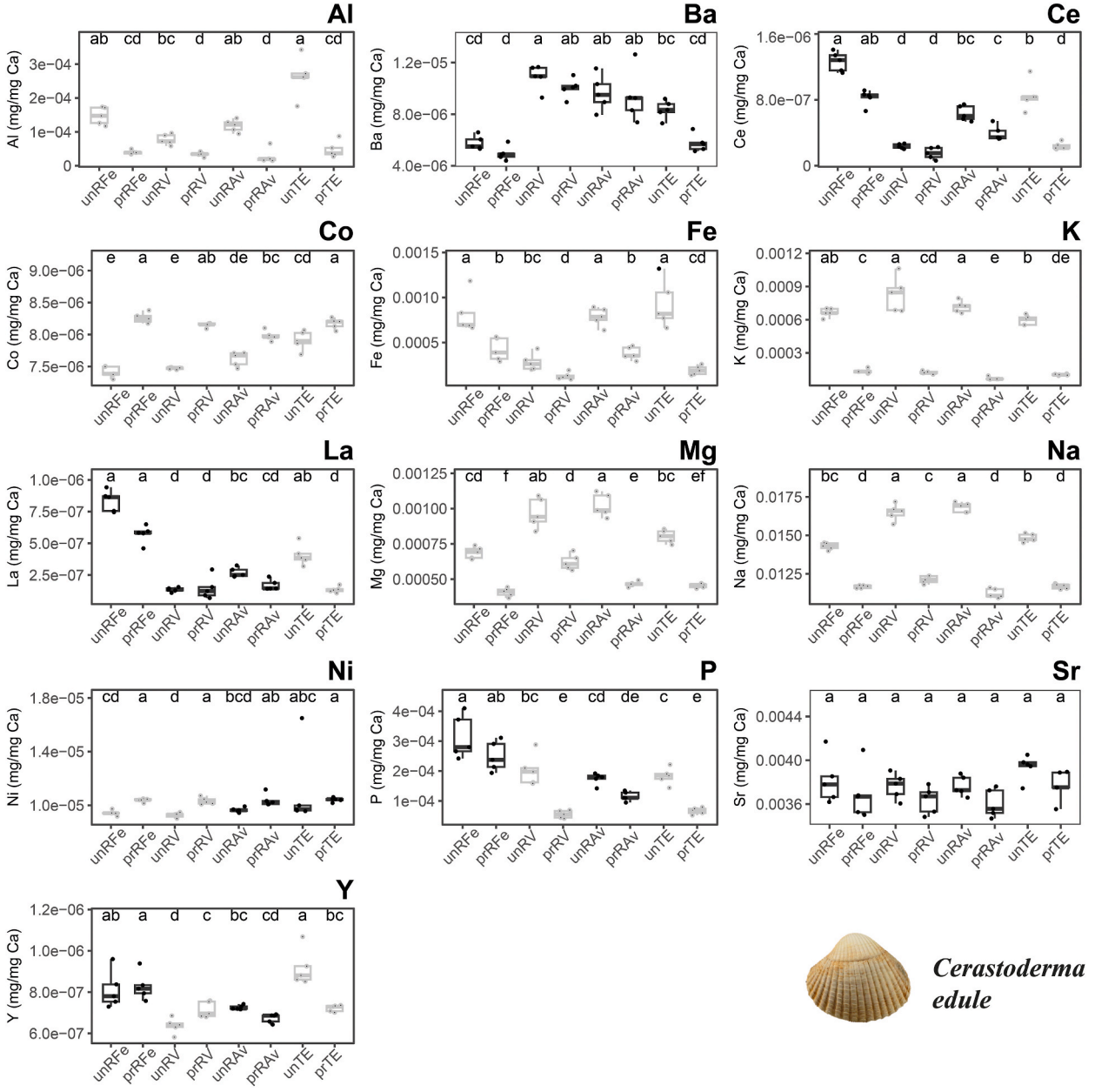
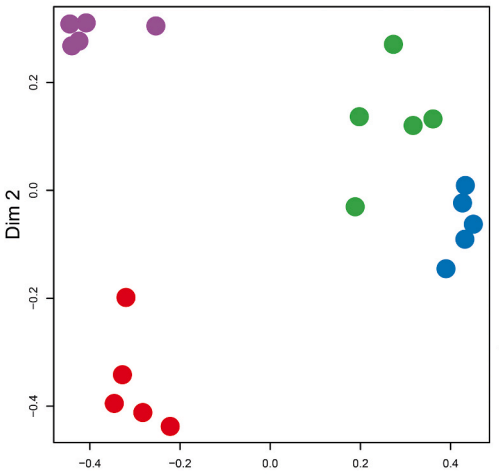


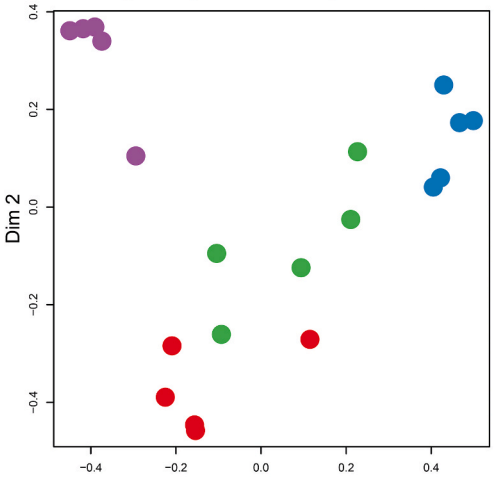
Fig. 3. Element-to-Ca ratios (mg/mg Ca) of *Cerastoderma edule* shells (pretreated and untreated) from four locations along the NW and W Iberian coast: untreated Ría de Ferrol (unRFe), pretreated Ría de Ferrol (prRFe), untreated Ría de Vigo (unRV), pretreated Ría de Vigo (prRV), untreated Ria de Aveiro (unRAv), pretreated Ria de Aveiro (prRAv), untreated Tagus estuary (unTE), and pretreated Tagus estuary (prTE). Different statistical letters (a, b, c, d, e, and f) denote significant differences between sampling sites at $p < 0.05$. Significant differences ($p < 0.05$) between treatments (pretreated and untreated) within the same location are highlighted with grey boxplots.

A. *R. phillipinarum* - untreated shells



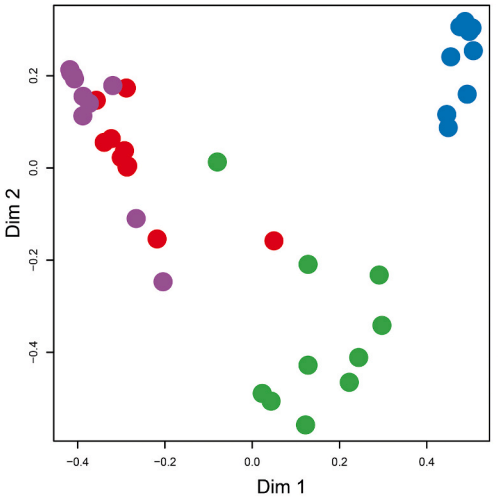
	Predicted Groups				
Original	**unRFe**	**unRV**	**unRAv**	**unTE**	**% Correct**
unRFe	5	0	0	0	100
unRV	0	5	0	0	100
unRAv	0	0	5	0	100
unTE	0	0	0	5	100
Total					**100**

B. *R. phillipinarum* - pretreated shells



	Predicted Groups				
Original	**prRFe**	**prRV**	**prRAv**	**prTE**	**% Correct**
prRFe	4	0	1	0	80
prRV	0	5	0	0	100
prRAv	1	0	4	0	80
prTE	0	0	0	5	100
Total					**90**

C. *R. phillipinarum* - untreated and pretreated shells grouped by location



	Predicted Groups				
Original Group	**RFe**	**RV**	**RAv**	**TE**	**% Correct**
RFe	9	0	1	0	90
RV	0	10	0	0	100
RAv	1	0	9	0	90
TE	0	0	0	10	100
Total					**95**

Ruditapes philippinarum

(caption on next page)

Fig. 4. Multidimensional scaling (MDS) ordinations of proximity scores and classification tables from Random Forest classifiers based on elemental fingerprints of *Ruditapes philippinarum* shells collected from four locations along the NW and W Iberian coasts. Untreated shells model: untreated Ría de Ferrol (unRFe), untreated Ría de Vigo (unRV), untreated Ria de Aveiro (unRAv), and untreated Tagus estuary (unTE); Pretreated shells model: pretreated Ría de Ferrol (prRFe), pretreated Ría de Vigo (prRV), pretreated Ria de Aveiro (prRAv), and pretreated Tagus estuary (prTE); All samples model: Ría de Ferrol (RFe), Ría de Vigo (RV), Ria de Aveiro (RAv), and the Tagus estuary (TE).

3. Results and discussion

3.1. Elemental fingerprints

Seventeen elements in *R. philippinarum* and thirteen in *C. edule* shells presented concentrations consistently above the ICP-MS detection limits (Figs. 2 and 3). PERMANOVA revealed a significant interaction for both species (location × treatment; $p = 0.0047$ *for R. philippinarum and* $p < 0.0001$ for *C. edule*) (Tables S2 and S3). The pair-wise comparisons (within location) revealed the existence of significant differences between the elemental fingerprints of pretreated and untreated shells for all locations, with p ranging from 0.0001 to 0.0011 for *R. philippinarum* and 0.0001 and 0.0007 for *C. edule* (Tables S2 and S3). Regarding individual elemental ratios, eight of them (Ag/Ca, Br/Ca, Cl/Ca, La/Ca, Mn/Ca, Nd/Ca, P/Ca, and Sr/Ca) in *R. philippinarum* and two of them (Ba/Ca and Sr/Ca) in *C. edule* shells presented no significant differences in all comparisons between pretreated and untreated shells within the same location (Figs. 2 and 3). However, several ratios from pretreated and untreated shells were significantly different within the same locations for both bivalve species surveyed (Figs. 2 and 3); Mg/Ca, Co/Ca, and Ni/Ca differed significantly in *R. philippinarum*, while Al/Ca, Co/Ca, Fe/Ca, K/Ca, Mg/Ca, and Na/Ca differed significantly in *C. edule* in four comparisons performed between pretreated and untreated shells within the same location; Cu/Ca, Na/Ca, and Y/Ca differed significantly in *R. philippinarum* in three comparisons; Al/Ca and K/Ca in *R. philippinarum* and Ni/Ca, P/Ca, and Y/Ca in *C. edule* differed significantly in two comparisons; and Fe/Ca in *R. philippinarum* and Cl/Ca and Na/Ca in *C. edule* presented significant differences in one comparison between pretreated and untreated shells within the same location (Figs. 2 and 3). The differences observed between pretreated and untreated shells for both the elemental fingerprints and individual elemental ratios-to-Ca are likely to result from the partial dissolution of calcium carbonate by laboratory-grade H_2O_2 [50,51], as previously reported for other aragonitic bivalve shells [*Arctica islandica*, 34] and (abiogenic) aragonites [35].

In general, the ratios presenting significant differences between pretreated and untreated shells within the same location decreased after H_2O_2 treatment, with Co/Ca, Ni/Ca, and Y/Ca being the exceptions, as these ratios displayed higher levels in the pretreated shells (Figs. 2 and 3). This reveals distinct degrees of elements leaching compared to Ca. However, the patterns found in this study should not be generalized for other aragonites because previous studies have reported contradictory effects resulting from the pretreatment process using H_2O_2 (30% w/v). For instance, Love & Woronow [35] reported general decreases in Fe/Ca, Sr/Ca, Mg/Ca, Mn/Ca, K/Ca, and Na/Ca in abiogenic aragonites, whereas Krause-Nehring et al. [34] described increases to Mg/Ca, Ba/Ca, and Mn/Ca in aragonitic bivalve shells (*Arctica islandica*).

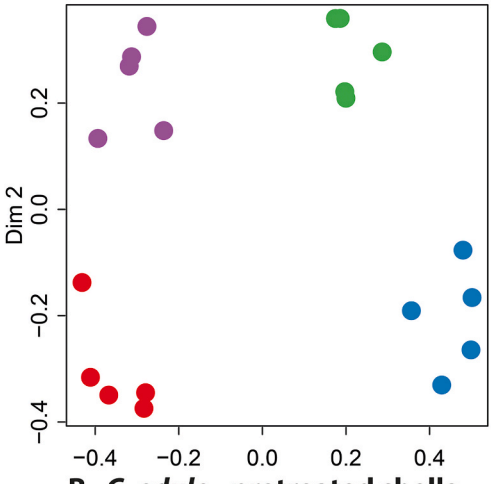
Overall, the pattern of each ratio driven by the H_2O_2 treatment was generally similar among locations for both species (Figs. 2 and 3). This indicates that, in this case, the differences in shells microstructures driven by local environmental conditions [52], or because they are different species [53], were not relevant in the leaching of elements.

3.2. Determination of geographic origin

The overall classification of the Random Forest models based on the EF of *R. philippinarum* and *C. edule* shells revealed high success rates for the models of shells untreated, treated and with both treatments (90%, 100% and 95; and 95%, 100 and 95%, respectively), which was supported by the good separation among sample groups in the MDS diagrams (Figs. 4A, B, and C and 5A, B, and C). For *R. philippinarum*, the highest classification success was obtained using untreated shells (100%, Fig. 4A), whereas for *C. edule*, it was obtained in the model using pretreated shells (100%, Fig. 5B). However, for both species, a narrow range of variation was observed among the three models, namely 90–100% for *R. philippinarum* and 95–100% for *C. edule* (Figs. 4 and 5). These results are in line with other geographic traceability studies that reported close accuracies of models based on the EF of pretreated and untreated shells with H_2O_2 (Table 1). Moreover, the high accuracies of the models of both species (95%) with pretreated and untreated shells grouped by location (Figs. 4C and 5C) revealed that the changes in the EF of shells promoted by the treatment with H_2O_2 do not impair the determination of the place of origin when using samples treated differently (i.e., pretreated and untreated shells) pooled in the same model. Although it should be noted that great caution must be taken when using samples processed with different pre-treatments prior ICP-MS analysis, this finding is particularly relevant under the present framework of data-sharing [11,54,55], as it shows the potential to combine data from studies that treated shells with or without H_2O_2.

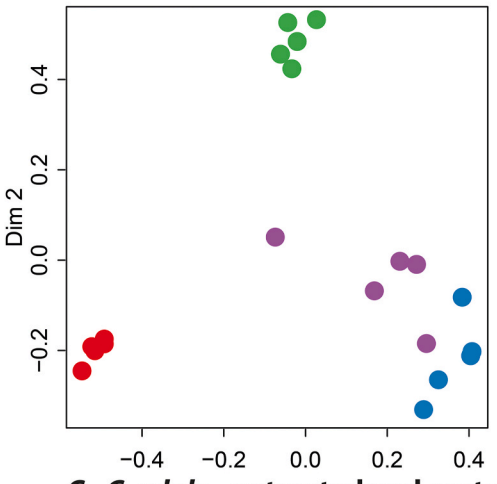
The high accuracy of all models (Figs. 4 and 5) indicates that the shell treatment with H_2O_2 prior to ICP-MS analysis can be suppressed, which agrees with Smith et al. [51] that recommended against any treatment to remove the organic matter from samples, thus avoiding preventing the occurrence of any changes in their mineralogy. This finding will improve the cost-efficiency of bivalve traceability tools, building upon previous methodological optimizations (e.g., [38]). The optimization here proposed is potentially relevant to traceability studies focusing on other bivalve species than *R. philippinarum* and *C. edule*, including species from other faunistic groups with calcareous structures (e.g., the capitula of goose barnacles, [56]). Moreover, these results may open the door to an analogous optimization in traceability studies using other biochemical signatures (e.g., stable isotopes) of bivalve shells, on which the treatment with H_2O_2 is also applied to avoid external interferences [57,58].

A. *C. edule* - untreated shells



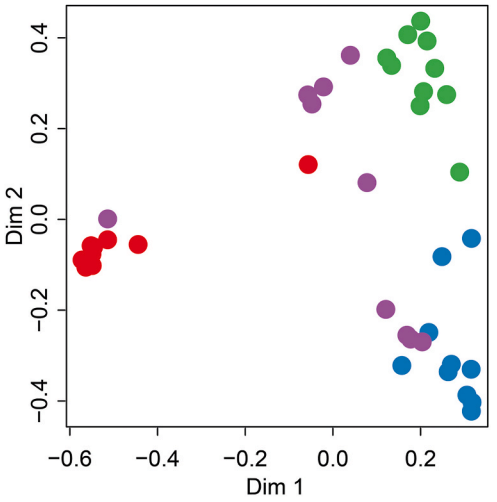
	Predicted Groups				
Original	**unRFe**	**unRV**	**unRAv**	**unTE**	**% Correct**
unRFe	5	0	0	0	100
unRV	0	5	0	0	100
unRAv	0	0	5	0	100
unTE	0	0	1	4	80
Total					**95**

B. *C. edule* - pretreated shells



	Predicted Groups				
Original	**prRFe**	**prRV**	**prRAv**	**prTE**	**% Correct**
prRFe	5	0	0	0	100
prRV	0	5	0	0	100
prRAv	0	0	5	0	100
prTE	0	0	0	5	100
Total					**100**

C. *C. edule* - untreated and pretreated shells grouped by location



	Predicted Groups				
Original Group	**RFe**	**RV**	**RAv**	**TE**	**% Correct**
RFe	9	0	0	1	90
RV	0	10	0	0	100
RAv	0	0	10	0	100
TE	1	0	0	9	90
Total					**95**

Cerastoderma edule

(caption on next page)

Fig. 5. Multidimensional scaling (MDS) ordinations of proximity scores and classification tables from Random Forest classifiers based on elemental fingerprints of *Cerastoderma edule* shells collected from four locations along the NW and W Iberian coasts. Untreated shells model: untreated Ría de Ferrol (unRFe), untreated Ría de Vigo (unRV), untreated Ria de Aveiro (unRAv), and untreated Tagus estuary (unTE); Pretreated shells model: pretreated Ría de Ferrol (prRFe), pretreated Ría de Vigo (prRV), pretreated Ria de Aveiro (prRAv), and pretreated Tagus estuary (prTE); All samples model: Ría de Ferrol (RFe), Ría de Vigo (RV), Ria de Aveiro (RAv), and the Tagus estuary (TE).

Table 1
Allocation accuracy of geographic traceability studies based on the elemental fingerprints of bivalve shells that assessed the accuracy of models using both pretreated and untreated (H_2O_2) samples.

Species	Allocation Accuracy (%)	Reference
Cerastoderma edule	90–100	This study
Ruditapes philippinarum	90–100	
Pecten maximus	94.9–97.5	[26]
Mytilus edulis	90%	[27]

The pretreatment with H_2O_2 requires at least three full days, and its elimination will streamline the methodology using EF of bivalve shells to confirm their geographic origin, with the most relevant consequence being a shorter timeframe spanning from analysis to the delivery of results. Moreover, as bivalves' traceability studies can at times encompass more than 700 samples (e.g., [23]) and the pretreatment of each *R. philippinarum* and *C. edule* valve requires approximately 20 ml of H_2O_2, the elimination of the H_2O_2 treatment can save more than 14 L of H_2O_2 per study. Therefore, the environmental impacts associated with the chemical residues produced from this procedure can be eliminated. Additionally, the economic benefits derived from not having to perform this treatment are also relevant, making the use of this tool more cost-efficient (potentially, the combined costs per study, including the purchase of laboratory-grade H_2O_2, would be approximately 200 euros), without counting the costs reduction associated with technician hand labor. It is also important to highlight that for larger bivalve species (e.g., oysters or scallops), the economic and environmental gains resulting from this methodological optimization will be even more relevant. On the other hand, the elimination of this procedure can also pose some risks. The pretreatment of bivalve shells with H_2O_2 aims to prevent interferences in the elemental fingerprints caused by the periostracum or other foreign organic matter (e.g., [22]). Therefore, skipping this pretreatment should only be considered after thoroughly cleaning mud, other organisms, and debris from the shells, as performed in the present study; otherwise, external interferences on the EF of shells can occur and bias the results from predictive models derived from their EF.

4. Conclusions

The present study revealed that the treatment of shells of *R. philippinarum* and *C. edule* with H_2O_2 promotes shifts in their EF, which, however, does not affect the accuracy of the models used to confirm the geographic origin of both species. While the method presented in this study demonstrates strengths, including the use of a highly sensitive analytical method (i.e., ICP-MS, [59]) with remarkable precision in allocating samples to their original locations (e.g., [20] and references therein), it is essential to acknowledge a limitation arising from the relatively low number of samples per location ($n = 5$) analyzed in this study. This introduces some level of uncertainty, which should be addressed in future studies by testing a larger set of samples. This study contributes to the optimization of seafood traceability tools based on the EF of calcareous structures by showing that a common methodological step (i.e., shell pretreatment with H_2O_2) can be dismissed. This will allow reducing the environmental impacts and economic costs associated with this procedure in future works, by decreasing the volume of residues produced during analysis and eliminating the need to purchase H_2O_2. More importantly, this new approach will speed up the delivery of results to authorities, seafood producers, or any other stakeholders, that want to scientifically support, or refute, claims on the geographic origin of seafood products to cope with existing legal procedures. Nevertheless, one must highlight that there is still room for optimizations of traceability tools used to confirm the geographic origin of seafood, such as the refinement of the minimum number of samples that have to be screened and the chemical elements that need to be fingerprinted.

Statement of informed consent, human/animal rights

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

Data availability statement

Data presented on this study is fully available as supplementary material.

CRediT authorship contribution statement

Renato Mamede: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Carla Patinha:** Writing – review & editing, Supervision, Methodology, Data curation. **Patrícia Martins:** Writing – review &

editing, Methodology. **Eduardo Ferreira da Silva:** Writing – review & editing. **Ricardo Calado:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Fernando Ricardo:** Writing – review & editing, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgements

This work was financially supported by project CITAQUA "Desenvolvimento do Projeto de Reforço do Polo de Aveiro (H4)", framed within Measure 10 of Investment TC-C10-i01 - Hub Azul - Rede de Infraestruturas para a Economia Azul, financed by the Recovery and Resilience Plan (PRR) and supported by Fundo Azul of the Portuguese Government". We also acknowledge the University of Aveiro, FCT/MEC for the financial support to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) through national funds and co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020. This study was also supported by GEOBIOTEC (UIDP/04035/2020) through national funds and co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020. We also acknowledge the project "Impacto e Consolidação em I&DT da Unidade de Investigação Química Orgânica, Produtos Naturais e Agroalimentares em áreas Agroalimentares e afins ICT_2009_02_005_2034" to finance the ICP-MS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25872.

References

[1] FAO, The State of World Fisheries and Aquaculture, Towards Blue Transformation, Rome, 2022, https://doi.org/10.4060/cc0461en.
[2] J. Guillen, F. Natale, N. Carvalho, J. Casey, J. Hofherr, J.N. Druon, G. Fiore, M. Gibin, A. Zanzi, J.T. Martinsohn, Global seafood consumption footprint, Ambio 48 (2019) 111–122, https://doi.org/10.1007/s13280-018-1060-9.
[3] A.C. Bosch, B. O'Neill, G.O. Sigge, S.E. Kerwath, L.C. Hoffman, Heavy metals in marine fish meat and consumer health: a review, J. Sci. Food Agric. 96 (2016) 32–48, https://doi.org/10.1002/jsfa.7360.
[4] M. Fox, M. Mitchell, M. Dean, C. Elliott, K. Christopher, The seafood supply chain from a fraudulent perspective, Food Secur 10 (2018) 939–963, https://doi.org/10.5505/turkhijyen.2018.75508.
[5] G.M. Luque, C.J. Donlan, The characterization of seafood mislabeling: a global meta-analysis, Biol. Conserv. 236 (2019) 556–570, https://doi.org/10.1016/j.biocon.2019.04.006.
[6] K. Gopi, D. Mazumder, J. Sammut, N. Saintilan, Determining the provenance and authenticity of seafood: a review of current methodologies, Trends Food Sci. Technol. 91 (2019) 294–304, https://doi.org/10.1016/j.tifs.2019.07.010.
[7] R. Mamede, F. Ricardo, A. Santos, S. Díaz, S.A.O. Santos, R. Bispo, M.R.M. Domingues, R. Calado, Revealing the illegal harvesting of Manila clams (*Ruditapes philippinarum*) using fatty acid profiles of the adductor muscle, Food Control (2020) 107368, https://doi.org/10.1016/j.foodcont.2020.107368.
[8] D. Menozzi, T.T. Nguyen, G. Sogari, D. Taskov, S. Lucas, J.L.S. Castro-Rial, C. Mora, Consumers' preferences and willingness to pay for fish products with health and environmental labels: evidence from five european countries, Nutrients 12 (2020) 1–22, https://doi.org/10.3390/nu12092650.
[9] J. Ramajal, D. Picard, J.L. Costa, F.B. Carvalho, M.B. Gaspar, P. Chainho, Amëijoa-japonesa, uma nova realidade no estuário do Rio Tejo: Pesca e pressão social e impacto socio-económico, in: L.C. Fonseca, A.C. Garcia, D.P. Pereira, M.A.C. Rodrigues (Eds.), Entre Rios e Mares Um Património Ambient. Histórias e Saberes, Tomo V Da Rede BrasPor, 2016, pp. 17–30.
[10] NIC, Global Implications of Illegal, Unreported, and Unregulated (IUU) Fishing, National Intelligence Council, 2016, pp. 1–21.
[11] EC, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, A European strategy for data, Brussels, 2020.
[12] EU, Regulation (EU) No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the common organisation of the markets in fishery and aquaculture products, amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC) No 104/2000, Off. J. Eur. Union 354 (2013) 1–21.
[13] M.C. Leal, T. Pimentel, F. Ricardo, R. Rosa, R. Calado, Seafood traceability: current needs, available tools, and biotechnological challenges for origin certification, Trends Biotechnol. 33 (2015) 331–336, https://doi.org/10.1016/j.tibtech.2015.03.003.
[14] C. Poulain, D.P. Gillikin, J. Thébault, J.M. Munaron, M. Bohn, R. Robert, Y.M. Paulet, A. Lorrain, An evaluation of Mg/Ca, Sr/Ca, and Ba/Ca ratios as environmental proxies in aragonite bivalve shells, Chem. Geol. 396 (2015) 42–50, https://doi.org/10.1016/j.chemgeo.2014.12.019.
[15] C. Richardson, Molluscs as archives of environmental change, Oceanogr. Mar. Biol. an Annu. Rev. 39 (2001) 103–164.
[16] A. del Rio-Lavín, J. Weber, J. Molkentin, E. Jiménez, I. Artetxe-Arrate, M.Á. Pardo, Stable isotope and trace element analysis for tracing the geographical origin of the Mediterranean mussel (*Mytilus galloprovincialis*) in food authentication, Food Control 139 (2022) 109069, https://doi.org/10.1016/j.foodcont.2022.109069.
[17] K. Gopi, D. Mazumder, J. Sammut, N. Saintilan, J. Crawford, P. Gadd, Combined use of stable isotope analysis and elemental profiling to determine provenance of black tiger prawns (*Penaeus monodon*), Food Control 95 (2019) 242–248, https://doi.org/10.1016/j.foodcont.2018.08.012.
[18] F. Ricardo, T. Pimentel, L. Génio, R. Calado, Spatio-temporal variability of trace elements fingerprints in cockle (*Cerastoderma edule*) shells and its relevance for tracing geographic origin, Sci. Rep. 7 (2017) 3475, https://doi.org/10.1038/s41598-017-03381-w.
[19] F. Ricardo, D. Gonçalves, T. Pimentel, R. Mamede, M.R.M. Domingues, A.I. Lillebø, R. Calado, Prevalence of phylogenetic over environmental drivers on the fatty acid profiles of the adductor muscle of marine bivalves and its relevance for traceability, Ecol. Indic. 129 (2021) 108017, https://doi.org/10.1016/j.ecolind.2021.108017.
[20] A. Santos, F. Ricardo, M.R.M. Domingues, C. Patinha, R. Calado, Current trends in the traceability of geographic origin and detection of species-mislabeling in marine bivalves, Food Control (2023) 109840, https://doi.org/10.1016/j.foodcont.2023.109840.

[21] M.O. Varrà, S. Ghidini, L. Husáková, A. Ianieri, E. Zanardi, Advances in troubleshooting fish and seafood authentication by inorganic elemental composition, Foods 10 (2021) 270, https://doi.org/10.3390/foods10020270.
[22] F. Ricardo, L. Genio, M.C. Leal, R. Albuquerque, H. Queiroga, R. Rosa, R. Calado, Trace element fingerprinting of cockle (*Cerastoderma edule*) shells can reveal harvesting location in adjacent areas, Sci. Rep. 5 (2015) 11932, https://doi.org/10.1038/srep11932.
[23] F. Ricardo, R. Mamede, A.L. Bruzos, S. Díaz, J. Thébault, E.F. da Silva, C. Patinha, R. Calado, Assessing the elemental fingerprints of cockle shells (*Cerastoderma edule*) to confirm their geographic origin from regional to international spatial scales, Sci. Total Environ. 814 (2022), https://doi.org/10.1016/j.scitotenv.2021.152304.
[24] J. Iguchi, Y. Takashima, A. Namikoshi, Y. Yamashita, M. Yamashita, Origin identification method by multiple trace elemental analysis of short-neck clams produced in Japan, China, and the Republic of Korea, Fish. Sci. 79 (2013) 977–982, https://doi.org/10.1007/s12562-013-0659-9.
[25] R. Mamede, A. Santos, S. Díaz, E. Ferreira da Silva, C. Patinha, R. Calado, F. Ricardo, Elemental fingerprints of bivalve shells (*Ruditapes decussatus* and *R. philippinarum*) as natural tags to confirm their geographic origin and expose fraudulent trade practices, Food Control 135 (2022) 108785, https://doi.org/10.1016/j.foodcont.2021.108785.
[26] L. Morrison, M. Bennion, S. Gill, C.T. Graham, Spatio-temporal trace element fingerprinting of king scallops (*Pecten maximus*) reveals harvesting period and location, Sci. Total Environ. 697 (2019) 134121, https://doi.org/10.1016/j.scitotenv.2019.134121.
[27] M. Bennion, L. Morrison, D. Brophy, J. Carlsson, J.C. Abrahantes, C.T. Graham, Trace element fingerprinting of blue mussel (*Mytilus edulis*) shells and soft tissues successfully reveals harvesting locations, Sci. Total Environ. 685 (2019) 50–58, https://doi.org/10.1016/j.scitotenv.2019.05.233.
[28] C.J.B. Sorte, R.J. Etter, R. Spackman, E.E. Boyle, R.E. Hannigan, Elemental fingerprinting of mussel shells to predict population sources and redistribution potential in the Gulf of Maine, PLoS One 8 (2013) 1–6, https://doi.org/10.1371/journal.pone.0080868.
[29] T. Forleo, A. Zappi, D. Melucci, M. Ciriaci, F. Griffoni, S. Bacchiocchi, M. Siracusa, T. Tavoloni, A. Piersanti, Inorganic elements in *Mytilus galloprovincialis* shells: geographic traceability by multivariate analysis of ICP-ms data, Molecules (2021) 2634, https://doi.org/10.3390/molecules26092634.
[30] A. Doubleday, J.C. Martino, C. Trueman, Harnessing universal chemical markers to trace the provenance of marine animals, Ecol. Indic. 144 (2022) 109481, https://doi.org/10.1016/j.ecolind.2022.109481.
[31] V.R. Bellotto, N. Miekeley, Trace metals in mussel shells and corresponding soft tissue samples: a validation experiment for the use of *Perna perna* shells in pollution monitoring, Anal. Bioanal. Chem. 389 (2007) 769–776, https://doi.org/10.1007/s00216-007-1420-y.
[32] L. Giordano, L. Ferraro, C. Caroppo, F. Rubino, F.P. Buonocunto, P. Maddalena, A method for bivalve shells characterization by FT-IR photoacoustic spectroscopy as a tool for environmental studies, MethodsX 9 (2022) 101672, https://doi.org/10.1016/j.mex.2022.101672.
[33] X. Wang, L. Li, Y. Zhu, X. Song, X. Fang, R. Huang, H. Que, G. Zhang, Aragonite shells are more ancient than calcite ones in bivalves: new evidence based on omics, Mol. Biol. Rep. 41 (2014) 7067–7071, https://doi.org/10.1007/s11033-014-3620-9.
[34] J. Krause-Nehring, A. Klgel, G. Nehrke, B. Brellochs, T. Brey, Impact of sample pretreatment on the measured element concentrations in the bivalve *Arctica islandica*, Geochem. Geophys. Geosyst. 12 (2011), https://doi.org/10.1029/2011GC003630.
[35] K.M. Love, A. Woronow, Chemical changes induced in aragonite using treatments for the destruction of organic material, Chem. Geol. 93 (1991) 291–301, https://doi.org/10.1016/0009-2541(91)90119-C.
[36] M. Bennion, L. Morrison, R. Shelley, C. Graham, Trace elemental fingerprinting of shells and soft tissues can identify the time of blue mussel (*Mytilus edulis*) harvesting, Food Control 121 (2021) 107515, https://doi.org/10.1016/j.foodcont.2020.107515.
[37] R. Mamede, F. Ricardo, D. Gonçalves, E. Ferreira da Silva, C. Patinha, R. Calado, Assessing the use of surrogate species for a more cost-effective traceability of geographic origin using elemental fingerprints of bivalve shells, Ecol. Indic. 130 (2021) 108065, https://doi.org/10.1016/j.ecolind.2021.108065.
[38] F. Ricardo, R. Mamede, R. Bispo, A. Santos, E. Ferreira da Silva, C. Patinha, R. Calado, Cost-efficiency improvement of bivalves shells preparation when tracing their geographic origin through ICP-MS analysis of elemental fingerprints, Food Control 118 (2020) 107383, https://doi.org/10.1016/j.foodcont.2020.107383.
[39] IPMA, Apanha e comercialização de moluscos bivalves, equinodermes, tunicados e gastrópodes marinhos vivos, Instituto Português do Mar e da Atmosfera, 2023, pp. 1–8.
[40] Pesca de Galicia, Datos xerais de pesca fresca de bivalvos no 2022, 2023. https://www.pescadegalicia.gal/Publicaciones/AnuarioPesca2022/Informes/1.1.2.html. (Accessed 4 December 2023).
[41] N. Tebble, British Bivalve Seashells. A Handbook for Identification, British Museum (Natural History), Alden Press, Oxford, 1976, p. 212.
[42] J. Humphreys, The introduction of the Manila clam to British coastal waters, Biologist 57 (2010) 134–139.
[43] L. Breiman, Random forests, Mach. Learn. 45 (2001) 5–32, https://doi.org/10.1201/9780367816377-11.
[44] L. Breiman, A. Cutler, A. Liaw, M. Wiener, "randomForest" 4.6–14: Breiman and Cutler's Random Forest for Classification and Regression, 2018, pp. 1–29, https://doi.org/10.1023/A:1010933404324.
[45] M.J. Anderson, R.N. Gorley, K.R. Clarke, PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods, PRIMER-E Ltd, Plymouth, UK, 2008.
[46] K.R. Clarke, R.N. Gorley, PRIMER v7: User Manual/tutorial, PRIMER-E, Plymouth, 2015.
[47] R. R Core Team, A Language and Environment for Statistical Computing, 2022, version 4.1.3.
[48] F. Mendiburu, "agricolae" 1.3-5, Statistical Procedures for Agricultural Research, 2021.
[49] H. Wickham, W. Chang, L. Henry, T.L. Pedersen, K. Takahashi, C. Wilke, D. Dunnington, K. Woo, H. Yutani, "ggplot 2" 3.3.5: create elegant data visualisations using the grammar of graphics. https://doi.org/10.1002/wics.147, 2021.
[50] S.J. Gaffey, C.E. Bronnimann, Effects of bleaching on organic and mineral phases in biogenic carbonates, J. Sediment. Petrol. 63 (1993) 752–754, https://doi.org/10.1306/d4267be0-2b26-11d7-8648000102c1865d.
[51] A.M. Smith, M.M. Key, Z.O.E.E. Henderson, V.C. Davis, D.J. Winter, Pretreatment for removal of organic material is not necessary for x-ray - diffraction determination of mineralogy in temperate skeletal carbonate, J. Sediment. Res. (2016) 1425–1433.
[52] N. Höche, E.O. Walliser, N.J. de Winter, R. Witbaard, B.R. Schöne, Temperature-induced microstructural changes in shells of laboratory-grown *Arctica islandica* (Bivalvia), PLoS One 16 (2021) 1–25, https://doi.org/10.1371/journal.pone.0247968.
[53] Z. Yao, M. Xia, H. Li, T. Chen, Y. Ye, H. Zheng, Bivalve shell: not an abundant useless waste but a functional and versatile biomaterial, Crit. Rev. Environ. Sci. Technol. 44 (2014) 2502–2530, https://doi.org/10.1080/10643389.2013.829763.
[54] W.K. Michener, Ecological data sharing, Ecol. Inform. 29 (2015) 33–44, https://doi.org/10.1016/j.ecoinf.2015.06.010.
[55] G. Popkin, Data sharing and how it can benefit your scientific career, Nature 569 (2019) 445–447, https://doi.org/10.1038/d41586-019-01506-x.
[56] R. Albuquerque, H. Queiroga, S.E. Swearer, R. Calado, S.M. Leandro, Harvest locations of goose barnacles can be successfully discriminated using trace elemental signatures, Sci. Rep. 6 (2016) 1–9, https://doi.org/10.1038/srep27787.
[57] G. Bianchini, V. Brombin, P. Carlino, E. Mistri, C. Natali, G.M. Salani, Traceability and authentication of manila clams from north-western adriatic lagoons using C and N stable isotope analysis, Molecules 26 (2021), https://doi.org/10.3390/molecules26071859.
[58] V. Brombin, C. Natali, G. Frijia, K. Schmitt, M. Casalini, G. Bianchini, Isotope geochemistry for seafood traceability and authentication: the northern adriatic manila clams case study, Foods 11 (2022), https://doi.org/10.3390/foods11193054.
[59] S.C. Wilschefski, M.R. Baxter, Inductively coupled Plasma mass spectrometry, Introd. Analyt. Aspects 40 (2019) 115–133.