Food Control 139 (2022) 109069

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Stable isotope and trace element analysis for tracing the geographical origin of the Mediterranean mussel (*Mytilus galloprovincialis*) in food authentication

Ane del Rio-Lavín [a,*], Jan Weber [b], Joachim Molkentin [c], Elisa Jiménez [a], Iraide Artetxe-Arrate [d], Miguel Ángel Pardo [a]

[a] AZTI, Food Research, Basque Research and Technology Alliance (BRTA). Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, 48160, Derio, Bizkaia, Spain
[b] National Reference Centre for Authentic Food, Max Rubner-Institute, Hermann-Weigmann-Str. 1, 24103, Kiel, Germany
[c] Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institute, Hermann-Weigmann-Str. 1, 24103, Kiel, Germany
[d] AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Txatxarramendi ugartea z/g, 48395, Sukarrieta, Bizkaia, Spain

ARTICLE INFO

Keywords:
Stable isotopes
Trace elements
Bivalves
Food authenticity
Traceability

ABSTRACT

Determining the geographical origin of seafood is crucial for regulators and fishing industries who seek to prevent commercial fraud, enforce food safety regulations and ensure high standards in sustainable fisheries management. Hence, we have investigated the potential of stable isotope ratio analysis (SIRA) and trace element fingerprinting (TEF), both individually and in combination, to trace the geographical origin of farmed *M. galloprovincialis* mussels harvested from eight different regions, located in the Mediterranean Sea, the European Atlantic coast and the Chilean Pacific coast. Particularly, carbon and nitrogen isotope ratios of mussel tissue (n = 179) and concentrations of 16 trace elements in shells (n = 100) were measured. Results show that significant differences exist in the chemical signatures of mussels harvested at different locations, and particularly among those belonging to different marine regions. Random forest (RF) classification method based on combined data (n = 64) revealed the best prediction accuracy correctly assigning 97% of individuals to their harvest location. The most relevant elements for provenance discrimination were $\delta^{13}C$, $\delta^{15}N$, Pb, Ba, Mn, and Al. Our results support the possibility for developing a reliable origin assignment tool based on stable isotopes and trace element analyses with global uses in seafood traceability and food safety.

1. Introduction

Global production of aquatic resources, including fisheries and aquaculture, reached a new peak in 2018, with a production of about 178.5 million tons of fish, molluscs, crustaceans and others, with an estimated total value of US$164.1 billion U.S. dollars (FAO, 2020). Within the fishery sector, mussels are an economically important product which is traded globally. The declaration of mussels has strict requirements and standards particularly when entering the European Union (EU) market, e.g., regarding the indication of origin or production method. In 2018, 17.7 million tons (USD 34.6 billion) of molluscs, mainly bivalves, were harvested, with consistent growth in production due to persistently high prices for bivalves in the past (FAO, 2020). Mussels of the species *Mytilus* spp. are globally almost exclusively farmed in aquacultures in China, the EU and Chile (EUMOFA, 2019). Within the widespread genus *Mytilus*, three species occur exclusively in the Northern Hemisphere (*M. trossulus*, *M. edulis*, *M. californianus*) and two in the Southern Hemisphere (*M. chilensis* and *M. platensis*), while *M. galloprovincialis* occurs on both hemispheres (Gaitán-Espitia, Quintero-Galvis, Mesas, & D'Elía, 2016; McDonald, Seed, & Koehn, 1991). The latter species, the Mediterranean mussel, is mainly endemic in Mediterranean Sea and Atlantic Ocean waters, while it is considered as an invasive species off North and South America, Australia, New Zealand, South Africa and East Asian coasts (Gardner, Oyarzún, Toro, Wenne, & Zbawicka, 2020; Hilbish et al., 2010; Larrain, Zbawicka, Araneda, Gardner, & Wenne, 2018; Westfall & Gardner, 2013; Zardi et al., 2018; Zbawicka, Trucco, & Wenne, 2018). Due to the overlapping habitats, natural inter-species hybridisation challenges the genetic authentication of *Mytilus* species for a valid declaration on the food market (Kijewski, Wijsman, Hummel, & Wenne, 2009).

* Corresponding author.
E-mail address: adelrio@azti.es (A. del Rio-Lavín).

https://doi.org/10.1016/j.foodcont.2022.109069
Received 16 February 2022; Received in revised form 13 April 2022; Accepted 29 April 2022
Available online 4 May 2022

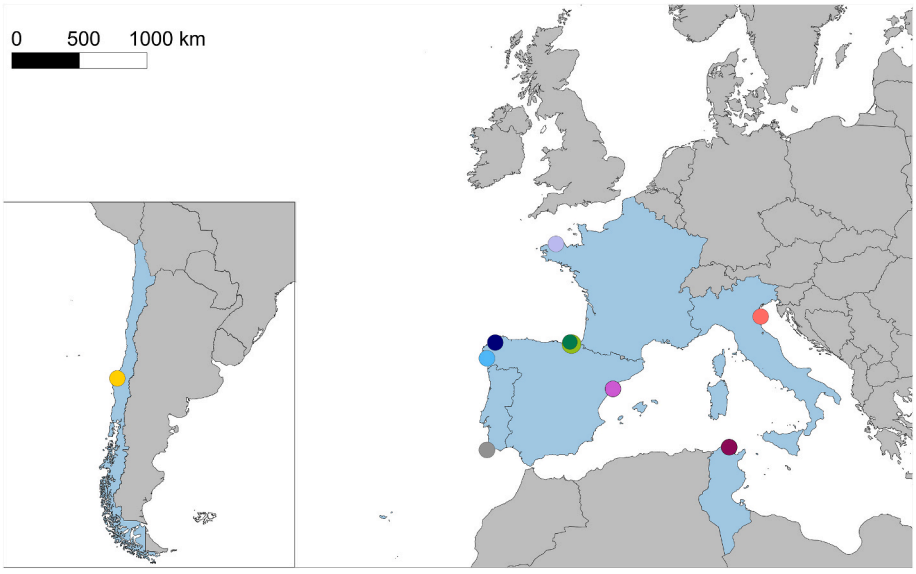

Fig. 1. Sampling locations of *M. galloprovincialis* and colour code; Atlantic Ocean area (LBFR, PBPT, Galicia region (AGES, SGES), Basque Country region (MEES, MUES)); the Mediterranean Sea area (DEES, GEIT, LBTN); and the South Eastern Pacific Ocean area (COCL). See Table 1 for definitions of abbreviations.

Considering the economic relevance of this extensively farmed species and its importance in the international trade, verification of its geographical origin is necessary for labelling, traceability and food safety purposes (Hixson, 2014; Luque & Donlan, 2019). Species identification is mandatory in the EU, as stated in Council Regulation (EC) No 1379/2013 (EU, 2013b). This includes the labelling of the commercial and scientific name, the production method and the geographical origin of the product (EU, 2013b). Furthermore, it should be noted that mussels from the region of Galicia ("Mexillón de Galicia", Spain) and Scardovari ("Cozza di Scardovi", Italy) have been authorized with the Protected Designation of Origin (PDO) in 2007 and 2013, respectively, due to their local history in mussel cultivation (EU, 2007; 2013a).

Mislabelling of the geographical origin is a common fraud, affecting the credibility of traders and deceiving consumers (Luque & Donlan, 2019). Innovative, accurate and sensitive analytical methods and approaches are essential to verify the compliance with the regulations in food authenticity. Methods used for seafood traceability include genetic markers (Giusti et al., 2020; Kappel & Schröder, 2020; Nielsen et al., 2012; Ogden & Linacre, 2015), fatty acid profiling (Cherifi, Chebil Ajjabi, & Sadok, 2018) and near infrared spectroscopy (J. Li, Yang, Li, Jabeen, & Shi, 2015; Van Cauwenberghe & Janssen, 2014). In addition, stable isotopes and elemental fingerprints have been recognized as particularly useful origin discriminators (L. Li, Boyd, & Sun, 2016).

Stable isotope ratio analysis (SIRA) has been established as a potent tool to trace the local provenance of bivalves and other aquatic species including fish, shrimps and sea cucumber (Deudero, Cabanellas, Blanco, & Tejada, 2009; Gustafson, Showers, Kwak, Levine, & Stoskopf, 2007; Kang et al., 2020; Ortea & Gallardo, 2015; Suh & Shin, 2013; X.; Zhao et al., 2019). Bivalves are sessile filter-feeders that assimilate in their tissues the isotopic composition from feeding sources such as phytoplankton, zooplankton, suspended particulate organic matter (POM) and other microorganisms from the water column (Purchon, 1968). Stable isotopes of carbon (δ13C) and nitrogen (δ15N) are well known geochemical tracers to study food web structures and ecosystem connectivity in aquatic environments (Peterson & Fry, 1987; Trueman & St John Glew, 2019). δ13C values of consumers reflect the source of energy that is produced by primary producers (e.g., phytoplankton, zooplankton, or POM), while δ15N values reflect the relative trophic position of the organism within the food web (Deniro & Epstein, 1981; Post, 2002). Although $\delta^{13}C$ and $\delta^{15}N$ composition of organisms primarily reflects their food absorbed (Deniro & Epstein, 1978, 1981), it has to be considered that isotope signatures of the mussels' food can be also influenced by local abiotic factors that may vary seasonally, such as sea surface temperature and dissolved inorganic carbon (DIC) in the case of carbon isotope signatures or changing nutrient conditions (e.g. eutrophic vs oligotrophic system) in the case of nitrogen isotope signatures (Briant et al., 2018).

In addition to considering seasonal variations in isotopic values, the selection of the sample material is critical, as different mussel tissues show different isotopic enrichment. While muscle tissue represents the average isotopic composition of the diet consumed over a longer period, the digestive gland and gonad, which have a high metabolic activity, primarily tend to reflect the recent diet (Ezgeta-Balić et al., 2014; Paulet, Lorrain, Richard, & Pouvreau, 2006). δ13C and δ15N in mussel tissue have already been used to trace the origin of *M. galloprovincialis* from the Balearic Islands and Yessow scallops (*Patinopecten yessoensis*) from the Yellow Sea (Deudero et al., 2009; X.; Zhao et al., 2019), anthropogenic pollution and sewage (Babaranti, Horn, Jowett, & Frew, 2019; Özdilek, Demir, & Gürkan, 2019), trophic level in the food web (Briant et al., 2018), and seasonal variation in the primary diet (Ezgeta-Balić et al., 2014; Page & Lastra, 2003). Accordingly, analysing δ13C and δ15N in the muscle of M. *galloprovincialis* while considering seasonal variation seems a promising approach for origin identification of internationally traded bivalves.

Over the last decades, trace elements have attracted much attention as natural fingerprints to trace the geographical origin of bivalves, both to identify population connectivity and to reconstruct past environmental changes (Becker, Fodrie, McMillan, & Levin, 2005; H. S.; Carson, 2010; Gomes et al., 2016; Honig, Etter, Pepperman, Morello, & Hannigan, 2020; Marali et al., 2017; Prendergast, Versteegh, & Schöne, 2017). As surrounding water chemistry and environmental conditions influence the incorporation of different elements into bivalve shells, variations in

Table 1
Samples used for TEF and SIRA analysis. MED = Mediterranean Sea area; ATL = Atlantic Ocean area; SEP = South Eastern Pacific Ocean area. *5 samples per season. ** Autumn missing.

Country	Area	Region	Sampling location	Acronym	n (TEF)	n (SIRA)	Seasonal sampling (SIRA)*	n (TEF + SIRA)
France	ATL	Brittany	Loquemeau	LBFR	10	17	yes**	8
Portugal	ATL	Algarve	Porto da Baleeira	PBPT	10	20	yes	6
Spain	ATL	Galicia (GAES)	Ria de Arousa	AGES	10	20	yes	5 + 5
			Ria de Betanzos-Sada	SGES	10	20	yes	
Spain	ATL	Basque Country (BQES)	Mendexa	MEES	10	20	yes	5 + 5
			Mutriku	MUES	10	20	yes	
Spain	MED	Catalonia	Delta del Ebro	DEES	10	20	yes	5
Italy	MED	Emilia-Romagna	Goro	GEIT	10	10	no	10
Tunisia	MED	Bizerte	Bizerte lagoon	LBTN	10	12	no	10
Chile	SEP	Biobío	Coliumo	COCL	10	20	yes	5
Total					*100*	*179*		*64*

the concentrations of selected elements can be used as natural markers to discriminate among groups of mussels grown under different environments (Becker et al., 2005; Strasser, Mullineaux, & Thorrold, 2008). Regarding traceability, several studies have demonstrated the capability of inductively coupled plasma-mass spectrometry (ICP-MS) based on trace element fingerprinting (TEF) of bivalve shells to assign samples to their origin (Bennion et al., 2019; Bennion, Morrison, Shelley, & Graham, 2021; Dunphy, Silva, & Gardner, 2015; Morrison, Bennion, Gill, & Graham, 2019; Ricardo et al., 2015; Ricardo, Pimentel, Génio, & Calado, 2017). However, these studies have limited their sampling sites to smaller spatial scales within the same country and none of them has focused on tracing the harvest location of the extensively worldwide distributed *M. galloprovincialis*.

The present study aims to assess the potential of using chemical signatures to trace the geographical origin of *M. galloprovincialis* mussels collected from ten commercial farms in the Mediterranean Sea, as well as on the European Atlantic coast and the Chilean Pacific coast. In addition to the two independent methodical approaches SIRA and TEF, we present a novel seafood traceability approach that combines organic stable isotope with inorganic trace element analysis.

2. Materials and methods

2.1. Sampling and preparation

Mytilus galloprovincialis samples for SIRA and TEF were collected between September 2018 and September 2019, with exception of Tunisian samples that were collected in January 2018. Collected mussel samples were immediately frozen (≤−20 °C) until further preparation. For this study, we defined the sampling seasons as follows: spring (April to May), summer (June to August), autumn (September to November) and winter (December to March). This division refers to the seasons in the Northern Hemisphere. Samples were obtained from 10 different locations (Fig. 1) in 6 different countries namely Spain, Portugal, France, Italy, Tunisia, and Chile (Table 1). The species identification of each individuum was verified genetically by a SYBR™ Green post-PCR melting curve analysis (Del Rio-Lavín, Jiménez, & Pardo, 2021). The sample set of the study consisted of a total of 179 soft tissue samples for SIRA and 100 shell samples for TEF; of which 64 individuals had both, the soft tissue and the shell, analysed (Table 1).

Mussel samples for SIRA (n = 179) were processed by removing the digestive gland from the tissue. The remaining soft tissue was cut into fine pieces of a few millimetres, mixed with purified water 1:2 (v/v) and chopped to a homogenous slurry using an Ultra-Turrax disperser (IKA, Germany). Finally, the slurry was lyophilized. Alternatively, the cut mussel tissue was directly dried in an oven set at 60 °C for 24h. Both methods worked equally well and showed no influence on the result. All dried tissue samples were mortared and stored at −20 °C until further analysis. Due to their low absolute lipid content with limited variations (Cherifi et al., 2018), lipids were not extracted from the tissue samples prior to SIRA (Deudero et al., 2009). Although compared to the defatted fraction a depletion in δ13C by −7.4‰ was analysed in the lipid fraction, the overall seasonal variation of lipids comprising 3% of total dry matter (10–13%) will just account for a maximum variation in δ13C of 0.2‰ in the composite samples. Thus, omitting the lipid correction had no significant effect on the variability of carbon results, but provides a simpler method for application in practice.

For trace element analysis, mussel shells (n = 100) were processed following a methodology similar to the one previously described by Bennion et al., 2019, Bennion et al., 2021, Morrison et al., 2019 and Ricardo et al., 2017. Briefly, all laboratory equipment used for sample preparation was cleaned using a 2–5% solution of DECON 90 (Decon™) for 2h, washed with running water, soaked in 10% of HNO_3 overnight and rinsed with MilliQ (Millipore) water. The valves were separated, and the organic tissue was removed using blades and tweezers. In order to remove any remaining organic matter and the periostracum, one valve per sample was soaked in 20 ml high-purity H_2O_2 (30% w/v) (Thermo Fisher Scientific) overnight and rinsed in MilliQ (Millipore) water three times.

2.2. Stable isotope ratio analysis and calibration

For sequentially δ13C and δ15N measurements, aliquots of 1.08 mg of dried tissue were weighed into tin capsules, using three replicates per individuum to check the repeatability of measurements as well as the homogeneity of samples. The wrapped samples were transferred into a Flash EA 1112 elemental analyser using an AS200 autosampler (Thermo Fisher Scientific, Waltham, MA/USA). The EA was equipped with a first reactor packed with chromium (III) oxide and silvered cobaltous oxide, where C and N were oxidized, supported by an oxygen pulse for 3 s, to CO2 and NOx at 1020 °C. Subsequently, the gases were transferred into a second reactor packed with copper wire, where NOx was reduced to N2 at 680 °C, followed by a water trap packed with magnesium perchlorate. The remaining gases N2 and CO2 were separated on a packed GC column at 45 °C at a continuous flow rate of 90 ml min-1 and transferred to a Delta Plus XL isotope-ratio mass spectrometer (Thermo Fisher Scientific) via a ConFlo III interface (Thermo Fisher Scientific). The isotope ratios are given in δ-notation expressed in ‰ referring to VPDB (Vienna Pee Dee Belemnite) and AIR, which define the zero point of the scale for δ13C and δ15N, respectively, and calculated according to the following equation using the software Isodat 2.0 (Thermo Fisher Scientific):

$$\delta X\ [‰] = [(R_{sample}/R_{standard}) - 1]$$

where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

The laboratory working standards, sucrose ($\delta^{13}C$) and urea ($\delta^{15}N$) (Merck, Germany), were calibrated by the following international secondary standards: NBS 22 ($\delta^{13}C_{VPDB}$ = −30.031‰), IAEA–CH–3

($\delta^{13}C_{VPDB} = -24.724‰$), and IAEA–CH–6 ($\delta^{13}C_{VPDB} = -10.449‰$) for carbon and IAEA-N1 ($\delta^{15}N_{Air} = +0.4‰$) and IAEA-N2 ($\delta^{15}N_{Air} = +20.3‰$) for nitrogen. The working standards were analysed in each sequence to validate the repeatability and to calibrate the monitoring gases carbon dioxide and nitrogen (Air Liquide, Germany). The standard deviation of consecutive monitoring gas analyses (n = 9) was ≤0.05‰, while triplicate sample measurements yielded a standard deviation of ≤0.15‰ for $\delta^{13}C$ and $\delta^{15}N$ (median 0.04‰, respectively).

2.3. Digestion and trace element analysis

From each shell, two fragments of approximately 10 mm^2 were selected and ground together: one fragment from the most recently formed edge and the other fragment located halfway between the umbo and the edge. Fragments were powdered using a ball mill MM 200 (Retsch, Hahn, Germany) with an agate grinding jar and digested in a close microwave device Mars 5 (CEM, Vertex, Barcelona, Spain) equipped with Teflon vessels and temperature controllers. The optimized microwave-assisted digestion procedure was carried out to achieve a shorter digestion time and dilution factor. Samples (around 200 mg) were directly placed into Teflon vessels, 10 ml of a concentrated HNO_3 (65%) (Agilent Technologies, Palo Alto, USA) was added to each flask and kept for 10 min at room temperature. Subsequently, the samples were digested following a one-stage digestion program (180 °C, 15 min). After digestion, the vessels were left to cool down and the resulting solution was diluted by the addition of ultrapure water obtained from Milli-Q Direct Water Purification System to reach a final volume of 50 ml.

The quantitative analysis of 16 trace elements (^{11}B, ^{27}Al, ^{47}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{111}Cd, ^{137}Ba, ^{208}Pb) was performed using an ICP-MS (7700x, Agilent Technologies, Palo Alto, USA) with a MicroMist micro-uptake glass concentric nebulizer (Glass Expansion, West Melbourne, Victoria, Australia). In order to reduce MO^+ formation in the plasma, the spray chamber was Peltier cooled at 2 °C. A standard quartz torch with 2.5 mm internal diameter injector was used. Finally, standard nickel cones (sample and skimmer) were used. The optimization of the ICP-MS conditions was achieved by adjusting the torch position and tuning for reducing oxide and doubly charged ion formation with a standard tuning solution containing 1.0 μg l^{-1} of ^{7}Li, ^{24}Mg, ^{59}Co, ^{89}Y, ^{140}Ce and ^{205}Tl in 1.0% HNO_3. This equipment includes a collision cell (He gas, ORS3 system, Agilent Technologies®) to discriminate spectral interferences for all the trace metals considered here with high performance. Alongside the samples, the European Reference Material ERM®-CE278K (powdered *M. edulis* mussel tissue) certified under the responsibility of the Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre was analysed. Before data analysis, elements which were not successfully recovered in the Certified Reference Materials were removed from all analyses (^{56}Fe and ^{78}Se were removed).

2.4. Statistical analysis

Basic statistical treatments of SIRA data were performed using JMP 15.1 (SAS Institute, USA). One-way ANOVA with post-hoc Tukey's HSD test was used to identify significant differences between the mean stable isotope values of individuals from different locations and on ocean area scale.

Trace element statistical analyses were performed using R software version 4.0.4 (R Core Team, 2021) and the statistical significance was determined at the alpha level of 0.05. Normality and homoscedasticity of each element individually were tested using Shapiro-Wilks (Steyn Jr. & Ellis, 2009) and Fligner-Killeen tests ("*stats*" package) respectively. Since trace element data did not meet parametric assumptions, Kruskal-Wallis test ("*stats*" package) was used to determine whether individual elemental concentrations varied among harvesting sites. When significant differences were found, post hoc pairwise comparisons were applied to identify the source of differences between harvest sites using Pairwise Wilcoxon Rank Sum Test ("*stats*" package). Statistical significance was determined based on adjusted p-values after the Benjamini-Hochberg correction (Benjamini & Hochberg, 1995).

Table 2
$\delta^{13}C$ and $\delta^{15}N$ all-season means ± standard deviation (SD) in *M. galloprovincialis* tissue. Within a column, letters identify significant differences ($p < 0.05$) between locations. MED = Mediterranean Sea area; ATL = Atlantic Ocean area; SEP = South Eastern Pacific Ocean area.

Location	Country	Area	$\delta^{13}C$		$\delta^{15}N$	
LBFR	France	ATL	−20.02 ± 0.39	c	+7.05 ± 0.68	c
PBPT	Portugal	ATL	−19.00 ± 0.75	b	+4.47 ± 0.39	e
AGES	Spain	ATL	−18.11 ± 0.54	a	+5.97 ± 0.38	d
SGES	Spain	ATL	−18.57 ± 0.58	ab	+6.16 ± 0.57	d
MEES	Spain	ATL	−21.16 ± 0.74	de	+4.87 ± 0.88	e
MUES	Spain	ATL	−20.81 ± 1.16	d	+5.57 ± 0.94	d
DEES	Spain	MED	−21.79 ± 0.51	e	+5.88 ± 0.58	d
GEIT	Italy	MED	−20.37 ± 0.19	cd	+5.79 ± 0.42	d
LBTN	Tunisia	MED	−21.77 ± 0.52	e	+10.17 ± 0.17	b
COCL	Chile	SEP	−19.86 ± 0.87	c	+11.77 ± 0.60	a

To test whether isotope and element fingerprinting could be used to successfully assign samples to their harvesting locations, the machine learning random forest (RF) classification method was used. RF uses a bootstrap aggregation algorithm to construct a multitude of decision trees (from randomly selected subset of training data) and combines the output of all the trees to obtain the most accurate prediction. This method was chosen as it has proven to have considerable advantages over many other multivariate classification analysis (Breiman, 2001), as it reduces overfitting problems in decision trees and also reduces the variance, resulting in an improvement of the accuracy. Additionally, random forest classification does not require the strict multivariate distributional and normality assumptions (Breiman, 2001) and performs best in the presence of skewness (Jones, Palmer, & Schaffler, 2017). In order to increase the number of individuals per location, data from sampling locations within the same region were combined and treated as a single group. RF was implemented using the R package "randomForest" (Liaw & Wiener, 2002) with the number of variables available for splitting at each tree node (mtry) limited to the square root of the number of variables in the analysis and using the Gini index (Cutler et al., 2007) to obtain the best elemental combination. This index measures the node impurity of the trees, and shows the probability of a new record being incorrectly classified at a given node in a decision tree based on the training data. So, the higher Mean Decrease in Gini index, the more important the variable is in classifying the data. In all cases, data were randomly split into a training dataset (75%) and a testing dataset (25%), and this procedure was randomly repeated 1000 times to avoid sampling effects. Each time, the rate of classification success (i.e., percentage of correct assignment of mussels to their harvesting region) was calculated, and mean accuracy values were extracted. Cohen's Kappa (κ) statistic was also calculated, which is a method that accounts for the agreement occurring just by chance. Values of κ range from 0 to 1, where 0 indicates that the RF resulted in no improvement over chance, and 1 indicates perfect agreement (Titus, Mosher, & Williams, 1984). Random forest was performed for trace element (n = 100) and stable isotope (n = 179) data individually, as well as for the combination of both measures coming from the same individual, to increase the number of predictor variables included. The resultant combined dataset was limited by the number of individuals for which both types of data were available (n = 64) (Table 1).

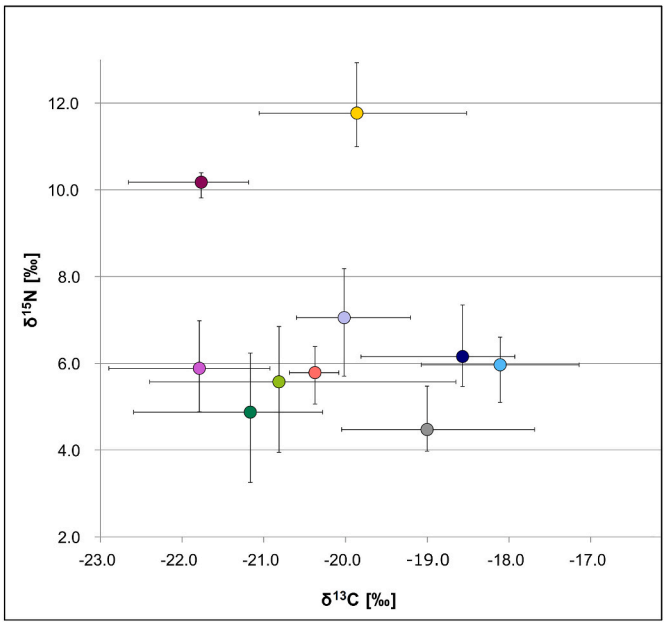

Fig. 2. Bivariate plot of $\delta^{13}C$ and $\delta^{15}N$ mean values with min/max ranges in *M. galloprovincialis* samples from different locations: Atlantic Ocean area (LBFR, PBPT, Galicia region (AGES, SGES), Basque Country region (MEES, MUES)); the Mediterranean Sea area (DEES, GEIT, LBTN); and the South Eastern Pacific area (COCL). See Table 1 for definitions of abbreviations.

3. Results and discussion

3.1. Stable isotope ratio analysis

3.1.1. Spatial variability

SIRA results of mean $\delta^{13}C$ and $\delta^{15}N$ values for the individual locations are summarized in **Table 2**. The lowest average $\delta^{13}C$ was found in the Spanish location DEES (-21.79 ± 0.51‰), while the highest average carbon isotope ratio occurred also in Spain at AGES (-18.11 ± 0.54‰). The highest average $\delta^{15}N$ was observed in Chile (COCL) with $+11.77 \pm 0.60$‰, while the lowest average of $+4.47 \pm 0.40$‰ occurred in Portugal (PBPT). The average $\delta^{13}C$ of Mediterranean sites (MED: 21.03 $\pm$ 0.93‰) is significantly more negative than that of Atlantic sites (ATL: 19.53 $\pm$ 1.45‰; $p < 0.001$) and the average of South East Pacific site (SEP: 19.86 $\pm$ 0.87‰) ($p < 0.001$), while no significant differentiation between ATL and SEP could be observed ($p < 0.283$). The mean $\delta^{15}N$ means differ significantly between all three marine areas (MED: +7.07 $\pm$ 1.74‰, ATL: +5.41 $\pm$ 0.92‰, SEP: +11.77 $\pm$ 0.60‰; $p < 0.0001$). Similarly, the present data show a great distinction between European and non-European samples, which is mainly driven by the significantly elevated $\delta^{15}N$ values of Chilean and Tunisian samples ($p < 0.0001$). Basically, our isotopic data obtained for *M. galloprovincialis* tissue are consistent with literature data except for the elevated $\delta^{15}N$ values in individuals from Chile and Tunisia (Briant et al., 2018; Deudero et al., 2009; Ezgeta-Balić et al., 2014; Page & Lastra, 2003; Rumolo et al., 2017; Özdilek et al., 2019). However, the higher $\delta^{15}N$ level in Chilean and Tunisian individuals is in a comparable range with other mussel species from certain regions at the French coast (Briant et al., 2018; S.; Dubois, Orvain, Marin-Léal, Ropert, & Lefebvre, 2007). Overall, the $\delta^{13}C$ values in this study are consistent with marine sources of organic matter (phytoplankton, algae and organic detritus) as the primary diet, being generally lower than those resulting from terrestrial sources (Simenstad & Wissmar, 1985). Similarly, marine specific nitrogen fixation processes were confirmed to be reflected in tissue $\delta^{15}N$ of the analysed mussels. The $\delta^{15}N$ is strongly influenced by the marine phytoplankton community, with N_2-fixing diazotrophs producing low $\delta^{15}N$ values (-2 to 0‰) by incorporating atmospheric N_2 as well as denitrifying organisms assimilating NO_3^- with enriched $\delta^{15}N$.

A bivariate plot of $\delta^{13}C$ vs $\delta^{15}N$ means and ranges at different locations (all-seasons) is illustrated in Fig. 2. The overall variation of -22.89 to -17.14‰ for $\delta^{13}C$ and $+3.49$ to $+12.50$‰ for $\delta^{15}N$ shows a considerable range within just one species, which is, however, in good agreement with literature data of mussels from various regions. $\delta^{13}C$ values in mussel tissue of the species *M. edulis* range from -22.0 to -17.1‰ and from -23.0 to -17.3 for *M. galloprovincialis,* while $\delta^{15}N$ values for these species range from $+4.3$ to $+12.5$‰ and $+3.2$ to $+4.9$‰, respectively (Briant et al., 2018; Deudero et al., 2009; S.; Dubois et al., 2007; Stanislas Dubois, Jean-Louis, Bertrand, & Lefebvre, 2007; Riera, 2007) Fig. 2 reveals that the origin of *M. galloprovincialis* can be identified just to a limited extent by SIRA. Especially the ranges of several European locations overlap and do not show significant differences in stable isotopic composition (cp. also Table 2). Hence, it can be concluded that the primary food sources and other environmental factors are largely comparable along the European coasts. Nevertheless, some regional specifics can be identified.

Within the Spanish *M. galloprovincialis* samples, comparable $\delta^{13}C$ and $\delta^{15}N$ values could be detected between the Galician samples AGES and SGES, and the samples MUES and MEES from the Basque Country, suggesting similar nutritional and nutrient conditions in the same region. Thus, based on the regionally consistent isotopic composition, the origin of PDO-protected *M. galloprovincialis* from Galicia can be verified by SIRA from most other regions, especially since unknown or potentially mislabelled mussels of other origins will mostly show different delta values (Fig. 2). However, samples from the Basque region (MUES, MEES) have a wide range of isotopic values, with especially $\delta^{13}C$ overlapping with almost all other European sites, which makes it difficult to assign samples to this origin unequivocally.

The $\delta^{13}C$ values in organic tissue of aquatic organisms are influenced by their primary diet. However, the diet of aquatic organisms can be influenced by anthropogenic pollution such as sewage, which may result in a potential interference in isotope values of mussels form coastal sites close to anthropogenic sources such as harbours or industries (Özdilek et al., 2019). Hence, the high variations in $\delta^{13}C$ at MUES, MEES (both Spain) and to a lesser extent at PBPT (Portugal) may reflect an anthropogenic overprint, as these sampling locations are nearby harbours, large coastal cities or estuaries. As discussed below, these fluctuations could also include a seasonal variability of the environment including ocean currents.

Comparable to the elevated $\delta^{15}N$ in Chilean mussels found in this study, several authors identified enhanced $\delta^{15}N$ values also in different marine species at the coasts of South America (Carrera & Gallardo, 2017; Espinoza et al., 2017). At the western coast of South America, the productive upwelling system of the Humboldt current causes ^{15}N enriched isotopic values, while beyond that, N_2-fixation by diazotrophs appears to have minor influence (Somes et al., 2010). The significantly elevated $\delta^{15}N$ values in the Tunisian samples are in contrast to an earlier study of mussel species from the Mediterranean Sea (Briant et al., 2018). In this study, the authors identified depleted $\delta^{15}N$ values in bivalve samples from the French coast bordering the Mediterranean Sea compared to individuals from the Atlantic Ocean and English Channel, probably due to the oligotrophic regime in Mediterranean Sea where N_2-fixation occurs (Briant et al., 2018; Liénart et al., 2017). However, we did not find significantly depleted $\delta^{15}N$ levels in individuals from the remaining Mediterranean coasts locations (DEES, GEIT) compared to individuals from locations bordering the Atlantic (PBPT, AGES, SGES, MEES and MUES) or the English Channel (LBFR) (Fig. 2). Accordingly, the elevated $\delta^{15}N$ from the Bizerte lagoon in Tunisia (LBTN) may rather be attributed to anthropogenic pollution. Several studies identified various signs of chemical contamination from agriculture, industry, shipping and urbanisation in the lagoons' sediments, but also in

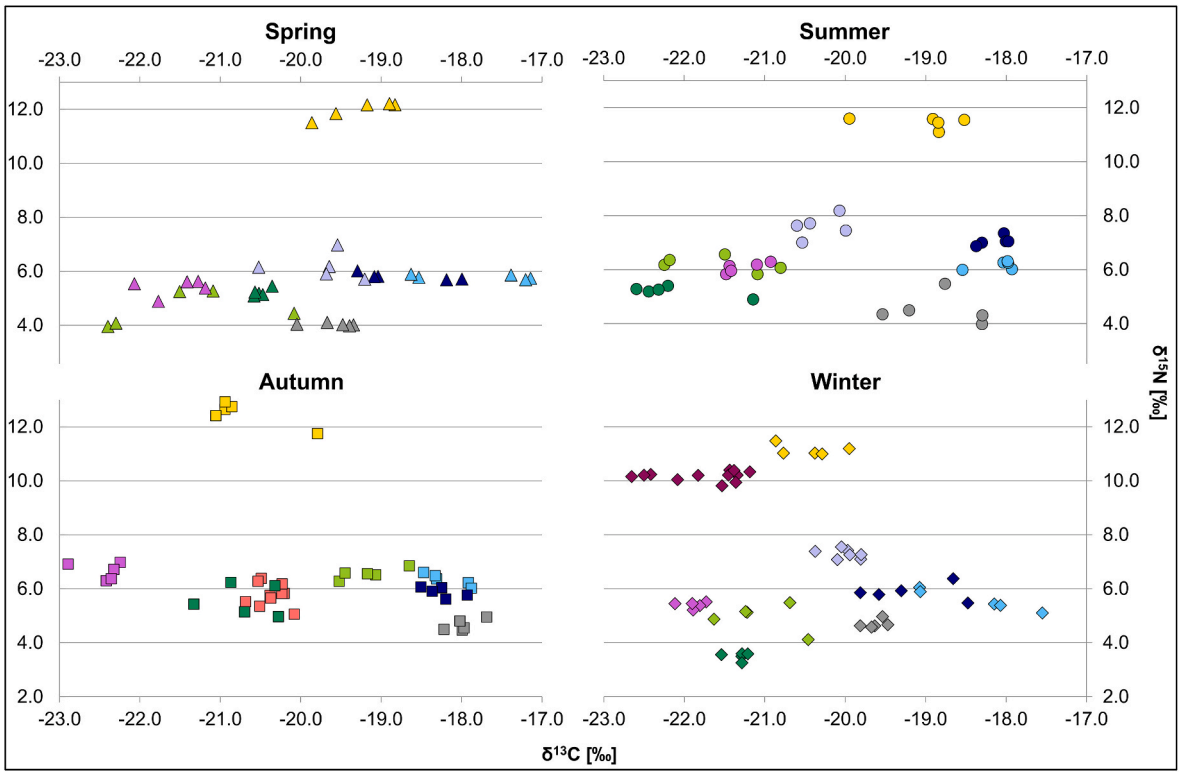

Fig. 3. Seasonal distribution of individual $\delta^{13}C$ and $\delta^{15}N$ values in *M. galloprovincialis* (n = 179). Each point represents an individual mussel belonging to one of the harvesting locations in the Atlantic Ocean area (LBFR, PBPT, Galicia region (AGES, SGES), Basque Country region (MEES, MUES)); the Mediterranean Sea area (DEES, GEIT, LBTN); and the South Eastern Pacific area (COCL). See Table 1 for definitions of abbreviations.

organisms thriving in the Bizerte lagoon (Barhoumi et al., 2014, 2016; Ben Ameur et al., 2013).

3.1.2. Seasonal variability

Seasonal fluctuations in carbon and nitrogen isotopic values of different organisms and food sources have been reported frequently, depending not only on the seasonal climate variability, but also on diet and feeding strategies (Bahar et al., 2008; Ezgeta-Balić et al., 2014; McCutchan & Lewis, 2001; Molkentin & Giesemann, 2010; X.; Zhao et al., 2019). As shown above, an exact geographical differentiation of the mussels is not feasible due to the high variation in $\delta^{13}C$ and $\delta^{15}N$ occurring at individual locations (Fig. 2). However, when considering the stable isotopic data on a seasonal basis, a more accurate discrimination between the different locations was achieved (Fig. 3). There is a considerable improvement in the distinction among the European sampling locations associated with a marginal overlap in the seasonal isotopic ranges. Nevertheless, no distinct trend in isotopic variation over the year could be observed for both elements.

It seems that the seasonal variation in isotopic values at individual locations is more affected by $\delta^{13}C$ than by $\delta^{15}N$. Highest seasonal variation in $\delta^{13}C$ is observed at MUES, with a difference of 3.75‰ (spring vs autumn), while the maximum $\delta^{15}N$ variation, 2.98‰, is found at MEES (winter vs autumn). In comparison, seasonal variations in AGES and SGES are rather limited, which is again favourable for the PDO detection in Galicia.

Several studies have detected significant seasonal differences in stable isotope composition of aquatic organisms due to seasonal food preferences, food availability or climatic factors (Chaguri et al., 2015; Sant'Ana, Ducatti, & Ramires, 2010; X.; Zhao et al., 2019). Potential environmental factors influencing the isotopic mussel composition may be seen at the Ebro delta (DEES). Samples from this location are highly influenced by the seasonal variations of the Ebro river discharge and varying water conditions over the year, which in turn varies depending on the climate (Ibañez, Pont, & Prat, 1997). Additionally, seasonal variations of the thriving phytoplankton community within the Ebro delta may be reflected in the mussels' stable isotopic composition. Phytoplankton biomarkers from October were reported to be more depleted in $\delta^{13}C$ in comparison to samples from June (Gómez-Gutiérrez, Tolosa, Jover, Bayona, & Albaigés, 2011), which is in accordance with the lowest $\delta^{13}C$ values found in DEES individuals of *M. galloprovincialis* from autumn.

The Chilean coast is strongly affected by seasonal disturbances within the Humboldt Current (Thiel et al., 2007). In contrast to the northern Chilean coast, where there is lower productivity associated with more stable conditions, a highly productive seasonal upwelling occurs in south-central Chile, where our samples were collected from (Montecino & Lange, 2009; Thomas, Carr, & Strub, 2001). Both $\delta^{13}C$ and $\delta^{15}N$ values from COCL are subjected to fluctuations throughout the year, being more pronounced in carbon isotopes (Fig. 3). Since the Humboldt Current is weaker in the Chilean summer, which corresponds to the Northern Hemisphere winter description in Fig. 3, $\delta^{15}N$ of the mussels is correspondingly lower then, due to the lack of the Humboldt increasing effect (Espinoza et al., 2017). Inter-annual scale events as the El Niño in the Southern Hemisphere have significant impact of the local nitrogen and carbon cycle, which in turn have an impact on the local phytoplankton and zooplankton community, which is the primary food of mussels (Montecino & Lange, 2009) Since the mussels used in this study were harvested between Northern Hemisphere winter 2018/19

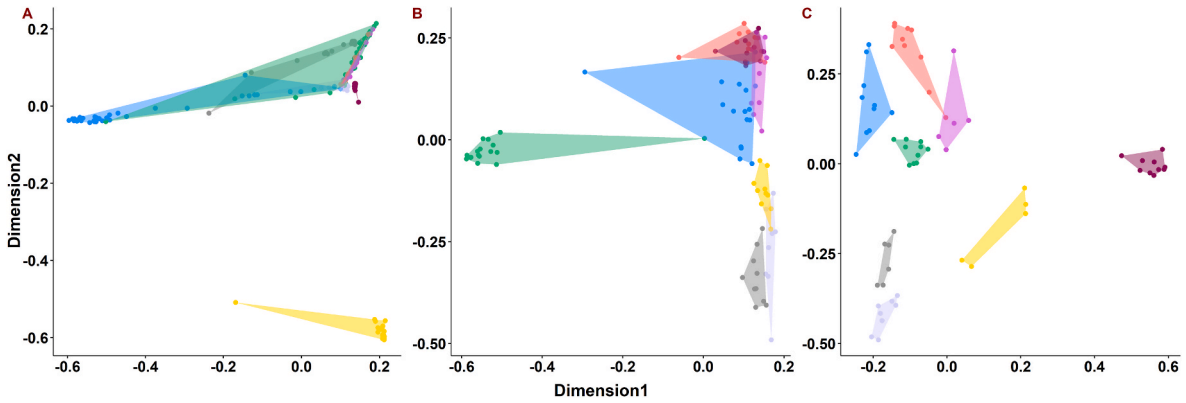

Fig. 4. Multidimensional scaling (MDS) of proximity scores from each of the random forest classifications based on (A) $\delta^{13}C$ and $\delta^{15}N$ values of soft tissues (n = 179), (B) fourteen trace element concentrations of shells (n = 100) and (C) the combination of both chemical signatures (n = 64). Each point represents an individual *M. galloprovincialis* mussel belonging to one of the harvest locations in the Atlantic Ocean area (LBFR, PBPT, Galicia region (GAES), Basque Country region (BQES)); the Mediterranean Sea area (DEES, GEIT, LBTN); and the South Eastern Pacific area (COCL). See Table 1 for definitions of abbreviations.

and autumn 2019, and a weak El Niño occurred during the same period (NOAA, 2021), this sample set may not be fully representative neither for non-El Niño years, nor for strong El Niño years.

In summary, the SIRA results show that the isotopic composition of mussels of the species *M. galloprovincialis* from different locations is highly variable. However, the application of isotopic data in source authentication is limited by their seasonal variation. The pronounced seasonal variation in stable isotopic values within individual sampling locations results in overlapping ranges, making an accurate determination of origin challenging. Therefore, the application of stable isotope analysis to samples of unknown origin allows the exclusion of individual locations rather than the exact determination of a specific origin. However, knowledge of the sampling season can improve the resolution, thus allowing a more accurate designation of the origin to be made.

3.2. Trace element analysis

Concentrations of individual elements detected in *M. galloprovincialis* shells varied differently between harvest locations when analysing them individually (**Table S2**). Results show that among the fourteen elements considered in the final analysis, Ba, Mn, Pb and Al (Kruskal Wallis Test; p-value(Ba) = $1.471e^{-12}$, p-value(Mn) = $1.544e^{-10}$, p-value(Pb) = $7.373e^{-13}$, p-value(Al) = $2.862e^{-07}$) were the trace elements that varied most between sites, meaning these elements were the main drivers of the observed differentiation. Conversely, no significant differences were depicted for Ti, Co and As (Kruskal Wallis Test; p-value $p > 0.05$) nor for Cr and Cu concentrations when performing pairwise comparisons between harvesting sites (Pairwise Wilcoxon Rank Sum Tests, $p > 0.05$) (**Table S2**). The incorporation of these elements into the calcite structures like mussel shells can be influenced by a number of interacting factors, such as elemental concentrations in seawater, metabolic efficiency of the individual, or environmental conditions (temperature, salinity, pH and dissolved oxygen among others) (Klein, Lohmann, & Thayer, 1996; Poulain et al., 2015; L.; Zhao et al., 2020). For example, higher concentrations of Ba and Mn have previously been related with terrestrial runoff and it has been seen that their uptake could be affected by temperature (influencing Ba) and dissolved oxygen (affecting both Ba and Mn) (Broadaway & Hannigan, 2012; H. S.; Carson, 2010) whereas heavy metals like Pb and Al have been associated to anthropogenic impacts (H. S. Carson et al., 2013). Although the understanding of factors contributing to the variability of elements can be helpful, the combination of these factors affecting each of the coastal ecosystems generates a unique pattern that might allow an accurate identification of the geographic origin, which is the focus of the present study.

3.3. Origin assignment: a combined approach

For the joint evaluation of SIRA and TEF data, locations within the same region were grouped together into BQES for Basque Country (in the case of MEES and MUES) or GAES for Galicia (in the case of AGES and SGES) regions (**Table 1**). The random forest model showed different classification success assigning individuals to their harvest region depending on the variables analysed. When the model was run based on trace element fingerprints of shells, mussels were assigned to their local harvest origin with an overall accuracy of 87.8% ($\kappa = 0.93$) (Table S1 and Fig. 4B). According to the Gini index, the most important elements for discriminating individuals between regions were Pb, Ba, Mn, and Al, which was expected as they were the elements that differed the most between origins in the statistical analysis. Shell elemental analyses have previously shown to be useful to assign bivalve species to their geographical origin using different classification approaches. Using discriminant function analysis (DFA), Becker et al. (2005) correctly assigned 56% of *M. californianus* and *M. galloprovincialis* mussels to six sampling points located in Southern California (reaching 90% of success when grouping samples in two regions) and Dunphy et al. (2011) correctly assigned 69% of *Perna canaliculus* individuals to six sampling points in northern New Zealand (reaching 63–100% when grouping samples into two regions). Performing an analysis of similarity, Ricardo et al. (2015) obtained 92% of classification success assigning *Cerastoderma edule* individuals to six sampling points in Portugal. Recently, by applying the machine learning RF classification, Bennion et al. (2019) correctly assigned 90% of *M. edulis* individuals to four sampling points in the west coast of Ireland and Morrison et al. (2019) achieved 97.5% of success assigning *Pecten maximus* to three sampling points in Ireland. These two last studies reached 100% of correctly assigned individuals to their origin when analysing elemental signature of both, tissue and shells. On this basis, in this present study, we have proved the potential of TEF of shells at higher spatial scales, correctly classifying 87.8% of *M. galloprovincialis* individuals to 8 different regions located in 6 different European countries. Using the stable isotopic composition of the tissue alone, results showed an overall classification success of 80.5% ($\kappa = 0.81$) to their harvest region (Table S1 and Fig. 4A). Mussels from LBTN and COCL show the highest classification accuracy, which is consistent with the pattern observed in the bivariate plot of δ13C and δ15N (Fig. 2).

To increase the likelihood of classification success, a combined dataset of trace element concentrations of shells and stable isotope ratios of muscle was used. The classification success of the random forest analysis ranged from 76% to 100% ($\kappa = 0.98$) depending on the

Table 3
Random forest classification success rates (%) in assigning farmed *M. galloprovincialis* mussels to their harvest region based on a combination of all trace element and stable isotope ratios. Assignment results using only the most important predictor variables for the assignment (δ13C, δ15N, Pb, Ba, Mn, and Al) are shown in brackets. See Table 1 for definitions of abbreviations.

		ATL				MED			SEP
		LBFR	PBPT	GAES	BQES	DEES	GEIT	LBTN	COCL
ATL	***LBFR***	**99** (100)	8 (2)	0	0	0	0	0	6 (2)
	PBPT	1 (0)	**92** (98)	0	0	0	0	0	0
	GAES	0	0	**96** (95)	0	0	0	0	0
	BQES	0	0	0	**97** (99)	2 (2)	0	0	0
MED	***DEES***	0	0	0	3 (1)	**76** (84)	0 (1)	0	0
	GEIT	0	0	4 (5)	0	22 (13)	**100** (99)	0	0
	LBTN	0	0	0	0	1 (1)	0	**100** (100)	2 (2)
SEP	***COCL***	0	0	0	0	0	0	0	**92** (95)

sampling region. Individuals collected from LBFR (France), BQES (Basque Country, Spain), GAES (Galicia, Spain), GEIT (Italy) and LBTN (Tunisia) displayed the highest percentages of correct classification (>96%) (Table 3 and Fig. 4C). The most important predictor variables included both stable isotope ratios (δ13C and δ15N) as well as the previously mentioned four trace elements (Pb, Ba, Mn and Al). Indeed, the combination of these six variables proved to be the most successful approach, obtaining an overall classification accuracy of 97% ($\kappa = 0.98$) This means that the number of variables analysed in future studies could be potentially reduced, which may result in a lower cost of the analysis and a reduced computational time.

The origin traceability tool presented here has proven to be useful to trace the origin of farmed Mediterranean mussels at the broadest geographical scale investigated to date, approaching a real usage scenario. Using chemical signatures for a species with numerous harvest areas like *M. galloprovincialis* can be a challenge, as the higher the number of harvest locations, the increased the likelihood of overlapping geochemical fingerprints. Nevertheless, the combination of stable isotopes and trace element fingerprints has demonstrated to be a successful approach, correctly assigning 97% of the individuals to their harvest region based on RF model.

4. Conclusions

This study reveals that the combination of stable isotope ratio analysis of tissue and trace element analysis of shells is an effective technique for determining the geographical origin of *M. galloprovincialis* mussels cultivated in ten different farms covering a large part of the geographical distribution. The separate use of each individual approach does not appear to be sufficient to fully discriminate the provenance, although certain locations can be excluded with certainty. This is not, however, the case of the PDO-protected *M. galloprovincialis* from Galicia, which could be well verified using SIRA alone. Overall, the present study reinforces the potential of using chemical signatures to trace seafood to their geographic origin. However, before this approach can be applied as a traceability tool by food control authorities, a reference collection of microchemical signatures should be first developed, which may require an intensive fieldwork at both temporal and spatial scales (Bennion et al., 2019; Ricardo et al., 2017). Besides, this methodology must be tested with a larger number of samples within each region and from additional farming locations, such as the United Kingdom, Ireland, and the eastern Mediterranean area, where this species is also actively cultivated. Our research paves the way for a reliable traceability tool development that will enforce food safety regulations and prevent commercial fraud.

CRediT authorship contribution statement

Ane del Rio-Lavín: Writing – original draft, Investigation, Visualization, Methodology, Formal analysis, Data curation, Writing – review & editing. **Jan Weber:** Writing – original draft, Formal analysis, Writing – review & editing. **Joachim Molkentin:** Conceptualization, Writing – original draft, Data curation, Methodology, Writing – review & editing. **Elisa Jiménez:** Conceptualization, Writing – review & editing. **Iraide Artetxe-Arrate:** Formal analysis, Data curation, Writing – review & editing. **Miguel Ángel Pardo:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Acknowledgements

Our study was carried out thanks to the financial support of the EU funded SEA-TRACES (EAPA_87/2016) INTERREG Atlantic Area project. The authors thank for the ICPMS MW service provided by SGIker (UPV/EHU/ERDF, EU). Ane del Rio is the recipient of a PhD grant from the Education Department of the Basque Government. Samples were kindly provided by Consello Regulador Mexillón de Galicia, IIM-CSIC, Fepromodel, Universidad de Chile, Muséum National D′ Histoire Naturelle and Indigo Rock Marine Research Station. Annette Hollmann, Birte Fischer-Kassebart and Bärbel Krumbeck are thanked for assistance in laboratory work and SIRA measurements. This manuscript is contribution number 1097 from AZTI, Food Research, Basque Research and Technology Alliance (BRTA).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.109069.

References

Babaranti, O., Horn, S., Jowett, T., & Frew, R. (2019). Isotopic signatures in Mytilus galloprovincialis and Ulva latuca as bioindicators for assessing discharged sewage effluent in coastal waters along Otago Peninsula, New Zealand. *Geology, Ecology, and Landscapes, 3*(1), 53–64. https://doi.org/10.1080/24749508.2018.1485079

Bahar, B., Schmidt, O., Moloney, A. P., Scrimgeour, C. M., Begley, I. S., & Monahan, F. J. (2008). Seasonal variation in the C, N and S stable isotope composition of retail organic and conventional Irish beef. *Food Chemistry, 106*(3), 1299–1305. https://doi.org/10.1016/j.foodchem.2007.07.053

Barhoumi, B., El Megdiche, Y., Clérandeau, C., Ameur, W. B., Mekni, S., Bouabdallah, S., et al. (2016). Occurrence of polycyclic aromatic hydrocarbons (PAHs) in mussel (Mytilus galloprovincialis) and eel (Anguilla anguilla) from Bizerte lagoon, Tunisia, and associated human health risk assessment. *Continental Shelf Research, 124*, 104–116. https://doi.org/10.1016/j.csr.2016.05.012

Barhoumi, B., LeMenach, K., Devier, M.-H., Ben Ameur, W., Etcheber, H., Budzinski, H., … Driss, M. R. (2014). Polycyclic aromatic hydrocarbons (PAHs) in surface sediments from the Bizerte Lagoon, Tunisia: Levels, sources, and toxicological significance. *Environmental Monitoring and Assessment, 186*(5), 2653–2669. https://doi.org/10.1007/s10661-013-3569-5

Becker[Bonnie, B. J. J.], Fodrie, F. J. [F. Joel], McMillan, P. A., & Levin, L. A. [Lisa A. (2005). Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: A precursor to invertebrate larval tracking. *Limnology & Oceanography, 50*(1), 48–61. https://doi.org/10.4319/lo.2005.50.1.0048

Ben Ameur, W., El Megdiche, Y., Eljarrat, E., Ben Hassine, S., Badreddine, B., Souad, T., et al. (2013). Organochlorine and organobromine compounds in a benthic fish (Solea solea) from Bizerte Lagoon (northern Tunisia): Implications for human exposure. *Ecotoxicology and Environmental Safety, 88*, 55–64. https://doi.org/10.1016/j.ecoenv.2012.10.021

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B, 57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bennion, M., Morrison, L., Brophy, D., Carlsson, J., Abrahantes, J. C., & Graham, C. T. (2019). Trace element fingerprinting of blue mussel (Mytilus edulis) shells and soft tissues successfully reveals harvesting locations. *The Science of the Total Environment, 685*, 50–58. https://doi.org/10.1016/j.scitotenv.2019.05.233

Bennion, M., Morrison, L., Shelley, R., & Graham, C. (2021). Trace elemental fingerprinting of shells and soft tissues can identify the time of blue mussel (Mytilus edulis) harvesting. *Food Control, 121*, 107515. https://doi.org/10.1016/j.foodcont.2020.107515

Breiman, L. (2001). Random forests. *Machine Learning, 45*(1), 5–32. https://doi.org/10.1023/A:1010933404324

Briant, N., Savoye, N., Chouvelon, T., David, V., Rodriguez, S., Charlier, K., … Knoery, J. (2018). Carbon and nitrogen elemental and isotopic ratios of filter-feeding bivalves along the French coasts: An assessment of specific, geographic, seasonal and multi-decadal variations. *The Science of the Total Environment, 613–614*, 196–207. https://doi.org/10.1016/j.scitotenv.2017.08.281

Broadaway, B. J., & Hannigan, R. E. (2012). Elemental fingerprints used to identify essential habitats: Nantucket bay scallop. *Journal of Shellfish Research, 31*(3), 671–676. https://doi.org/10.2983/035.031.0310, 6.

Carrera, M., & Gallardo, J. M. (2017). Determination of the geographical origin of all commercial hake species by stable isotope ratio (SIR) analysis. *Journal of Agricultural and Food Chemistry, 65*(5), 1070–1077. https://doi.org/10.1021/acs.jafc.6b04972

Carson, H. S. (2010). Population connectivity of the Olympia oyster in southern California. *Limnology & Oceanography, 55*(1), 134–148. https://doi.org/10.4319/lo.2010.55.1.0134

Carson, H. S., López-Duarte, P. C., Cook, G. S., Fodrie, F. J.[F. J.], Becker, B. J.[B. J.], DiBacco, C., et al. (2013). Temporal, spatial, and interspecific variation in geochemical signatures within fish otoliths, bivalve larval shells, and crustacean larvae. *Marine Ecology Progress Series, 473*, 133–148. https://doi.org/10.3354/meps10078

Chaguri, M. P., Maulvault, A. L., Nunes, M. L., Santiago, D. A., Denadai, J. C., Fogaça, F. H., … Marques, A. (2015). Different tools to trace geographic origin and seasonality of croaker (Micropogonias furnieri). *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 61*(1), 194–200. https://doi.org/10.1016/j.lwt.2014.11.006

Cherifi, H., Chebil Ajjabi, L., & Sadok, S. (2018). Nutritional value of the Tunisian mussel Mytilus galloprovincialis with a special emphasis on lipid quality. *Food Chemistry, 268*, 307–314. https://doi.org/10.1016/j.foodchem.2018.06.075

Cutler, D. R., Edwards, T. C., Beard, K. H., Cutler, A., Hess, K. T., Gibson, J., et al. (2007). Random forests for classification in ecology. *Ecology, 88*(11), 2783–2792. https://doi.org/10.1890/07-0539.1

Del Rio-Lavín, A., Jiménez, E., & Pardo, M.Á. (2021). SYBR-Green real-time PCR assay with melting curve analysis for the rapid identification of Mytilus species in food samples. *Food Control, 130*, 108257. https://doi.org/10.1016/j.foodcont.2021.108257

Deniro, M. J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta, 42*(5), 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

Deniro, M. J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta, 45*(3), 341–351. https://doi.org/10.1016/0016-7037(81)90244-1

Deudero, S., Cabanellas, M., Blanco, A., & Tejada, S. (2009). Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel Mytilus galloprovincialis. *Journal of Experimental Marine Biology and Ecology, 368*(2), 181–188. https://doi.org/10.1016/j.jembe.2008.10.008

Dubois, S.[S.], Orvain, F., Marin-Léal, J. C., Ropert, M., & Lefebvre, S.[S. (2007). Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. *Marine Ecology Progress Series, 336*, 151–160. https://doi.org/10.3354/meps336151

Dubois, S. [Stanislas], Jean-Louis, B., Bertrand, B., & Lefebvre[Sébastien], S. (2007). Isotope trophic-step fractionation of suspension-feeding species: Implications for food partitioning in coastal ecosystems. *Journal of Experimental Marine Biology and Ecology, 351*(1–2), 121–128. https://doi.org/10.1016/j.jembe.2007.06.020

Dunphy, B. J., Millet, M. A., & Jeffs, A. G. (2011). Elemental signatures in the shells of early juvenile green-lipped mussels (Perna canaliculus) and their potential use for larval tracking. *Aquaculture, 311*(1–4), 187–192. https://doi.org/10.1016/j.aquaculture.2010.12.016

Dunphy, B. J., Silva, C., & Gardner, J. P. (2015). *Testing techniques for tracing the provenance of green-lipped mussel spat washed up on Ninety Mile Beach.* https://doi.org/10.13140/rg.2.1.3379.3525. Unpublished.

Espinoza, P., Lorrain, A. [Anne], Ménard, F., Cherel, Y., Tremblay-Boyer, L., Argüelles, J., … Bertrand, A. (2017). Trophic structure in the northern Humboldt current system: New perspectives from stable isotope analysis. *Marine Biology, 164*(4). https://doi.org/10.1007/s00227-017-3119-8

EU. (2007). *Commision Regulation (EC) No 1050/2007 of 12 September 2007 registering certain names in the Register of protected designations of origin and protected geographical indications (Mejillón de Galicia or Mexillón de Galicia (PDO) — café de Colombia (PGI) — castagna Cuneo (PGI) — asparago Bianco di Bassano (PDO))*.

EU. (2013a). *Commission Implementing Regulation (EU) No 1200/2013 of 25 November 2013 entering a name in the register of protected designations of origin and protected geographical indications [Cozza di Scardovari (PDO)]*.

EU. (2013b). *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*.

EUMOFA. (2019). *Frische Muscheln in der EU*. Advance online publication. https://doi.org/10.2771/567624

Ezgeta-Balić, D., Lojen, S., Dolenec, T., Žvab Rožič, P., Dolenec, M., Najdek, M., et al. (2014). Seasonal differences of stable isotope composition and lipid content in four bivalve species from the Adriatic Sea. *Marine Biology Research, 10*(6), 625–634. https://doi.org/10.1080/17451000.2013.833338

FAO. (2020). The state of world fisheries and aquaculture 2020. Food and agriculture organization. https://doi.org/10.4060/ca9229en.

Gaitán-Espitia, J. D., Quintero-Galvis, J. F., Mesas, A., & D'Elía, G. (2016). Mitogenomics of southern hemisphere blue mussels (Bivalvia: Pteriomorphia): Insights into the evolutionary characteristics of the Mytilus edulis complex. *Scientific Reports, 6*, 26853. https://doi.org/10.1038/srep26853

Gardner, J. P. A., Oyarzún, P. A., Toro, J. E., Wenne, R., & Zbawicka, M. (2020). Phylogeography of Southern Hemisphere Blue Mussels of the Genus *Mytilus*: Evolution, Biosecurity, Aquaculture and Food Labelling, *Vols. 139–228. Oceanography and Marine Biology: An Annual Review, Volume 59*. Taylor & Francis.

Giusti, A., Tosi, F., Tinacci, L., Guardone, L., Corti, I., Arcangeli, G., et al. (2020). Mussels (Mytilus spp.) products authentication: A case study on the Italian market confirms issues in species identification and arises concern on commercial names attribution. *Food Control, 118*, 107379. https://doi.org/10.1016/j.foodcont.2020.107379

Gomes, I., Peteiro, L. G., Albuquerque, R.[R.], Nolasco, R., Dubert, J., Swearer, S. E., et al. (2016). Wandering mussels: Using natural tags to identify connectivity patterns among marine protected areas. *Marine Ecology Progress Series, 552*, 159–176. https://doi.org/10.3354/meps11753

Gómez-Gutiérrez, A., Tolosa, I., Jover, E., Bayona, J. M., & Albaigés, J. (2011). Origin and vertical dynamics of particulate organic matter in a salt-wedge estuary, the Ebro Delta, inferred from lipid molecular markers and compound-specific isotope carbon analysis. *Marine Chemistry, 126*(1–4), 269–280. https://doi.org/10.1016/j.marchem.2011.06.006

Gustafson, L., Showers, W., Kwak, T., Levine, J., & Stoskopf, M. (2007). Temporal and spatial variability in stable isotope compositions of a freshwater mussel: Implications for biomonitoring and ecological studies. *Oecologia, 152*(1), 140–150. https://doi.org/10.1007/s00442-006-0633-7

Hilbish, T. J., Brannock, P. M., Jones, K. R., Smith, A. B., Bullock, B. N., & Wethey, D. S. (2010). Historical changes in the distributions of invasive and endemic marine invertebrates are contrary to global warming predictions: The effects of decadal climate oscillations. *Journal of Biogeography, 37*(3), 423–431. https://doi.org/10.1111/j.1365-2699.2009.02218.x

Hixson, S. M. (2014). Fish nutrition and current issues in aquaculture: The balance in providing safe and nutritious seafood, in an environmentally sustainable manner. *Journal of Aquaculture Research & Development, 3*(3). https://doi.org/10.4172/2155-9546.1000234

Honig, A., Etter, R., Pepperman, K., Morello, S., & Hannigan, R. (2020). Site and age discrimination using trace element fingerprints in the blue mussel, Mytilus edulis. *Journal of Experimental Marine Biology and Ecology, 522*, 151249. https://doi.org/10.1016/j.jembe.2019.151249

Ibañez, C., Pont, D., & Prat, N. (1997). Characterization of the ebre and rhone estuaries: A basis for defining and classifying salt-wedge estuaries. *Limnology & Oceanography, 42*(1), 89–101. https://doi.org/10.4319/lo.1997.42.1.0089

Jones, C. M., Palmer, M., & Schaffler, J. J. (2017). Beyond Zar: The use and abuse of classification statistics for otolith chemistry. *Journal of Fish Biology, 90*(2), 492–504. https://doi.org/10.1111/jfb.13051

Kang, X., Zhao, Y., Shang, D., Zhai, Y., Ning, J., Ding, H., et al. (2020). Identification of the geographical origins of sea cucumbers in China: The application of stable isotope ratios and compositions of C, N, O and H. *Food Control, 111*, 107036. https://doi.org/10.1016/j.foodcont.2019.107036

Kappel, K., & Schröder, U. (2020). Difficulties in DNA barcoding-based authentication of snapper products due to ambiguous nucleotide sequences in public databases. *Food Control, 118*, 107348. https://doi.org/10.1016/j.foodcont.2020.107348

Kijewski, T., Wijsman, J. W., Hummel, H., & Wenne, R. (2009). Genetic composition of cultured and wild mussels Mytilus from The Netherlands and transfers from Ireland and Great Britain. *Aquaculture, 287*(3–4), 292–296. https://doi.org/10.1016/j.aquaculture.2008.10.048

Klein, R. T., Lohmann, K. C., & Thayer, C. W. (1996). Bivalve skeletons record sea-surface temperature and δ18O via Mg/Ca and 18O/16O ratios. *Geology, 24*(5), 415. https://doi.org/10.1130/0091-7613(1996)024<0415:BSRSST>2.3.CO, 2.

Larrain, M. A., Zbawicka, M. [Malgorzata], Araneda, C., Gardner, J. P. A., & Wenne, R. (2018). Native and invasive taxa on the Pacific coast of South America: Impacts on aquaculture, traceability and biodiversity of blue mussels (Mytilus spp.). *Evolutionary Applications, 11*(3), 298–311. https://doi.org/10.1111/eva.12553

Liaw, A., & Wiener, M. (2002). *Classification and regression by RandomForest* (pp. 18–22). Forest,23.

Liénart, C., Savoye, N., Bozec, Y., Breton, E., Conan, P., David, V., … Sultan, E. (2017). Dynamics of particulate organic matter composition in coastal systems: A spatio-temporal study at multi-systems scale. *Progress in Oceanography, 156*, 221–239. https://doi.org/10.1016/j.pocean.2017.03.001

Li[Li], L., Boyd, C. E., & Sun, Z. (2016). Authentication of fishery and aquaculture products by multi-element and stable isotope analysis. *Food Chemistry, 194*, 1238–1244. https://doi.org/10.1016/j.foodchem.2015.08.123

Li, J., Yang, D., Li, L. [Lan], Jabeen, K., & Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental Pollution, 207*, 190–195. https://doi.org/10.1016/j.envpol.2015.09.018

Luque, G. M., & Donlan, C. J. (2019). The characterization of seafood mislabeling: A global meta-analysis. *Biological Conservation, 236*, 556–570. https://doi.org/10.1016/j.biocon.2019.04.006

Marali, S., Schöne [Bernd, B. R. R.], Mertz-Kraus, R., Griffin, S. M., Wanamaker, A. D., Matras, U., et al. (2017). Ba/Ca ratios in shells of Arctica islandica —potential environmental proxy and crossdating tool. *Palaeogeography, Palaeoclimatology, Palaeoecology, 465*, 347–361. https://doi.org/10.1016/j.palaeo.2015.12.018

McCutchan, J. H., & Lewis, W. M. (2001). Seasonal variation in stable isotope ratios of stream algae. *SIL Proceedings, 27*(6), 3304–3307. https://doi.org/10.1080/03680770.1998.11902437, 1922-2010.

McDonald, J. H., Seed, R., & Koehn, R. K. (1991). Allozymes and morphometric characters of three species of Mytilus in the Northern and Southern Hemispheres// Allozymes and morphometric characters of three species ofMytilus in the Northern and Southern Hemispheres. *Marine Biology, 111*(3), 323–333. https://doi.org/10.1007/BF01319403

Molkentin, J., & Giesemann, A. (2010). Follow-up of stable isotope analysis of organic versus conventional milk. *Analytical and Bioanalytical Chemistry, 398*(3), 1493–1500. https://doi.org/10.1007/s00216-010-3995-y

Montecino, V., & Lange, C. B. (2009). The Humboldt Current System: Ecosystem components and processes, fisheries, and sediment studies. *Progress in Oceanography, 83*(1–4), 65–79. https://doi.org/10.1016/j.pocean.2009.07.041

Morrison, L., Bennion, M., Gill, S., & Graham, C. T. (2019). Spatio-temporal trace element fingerprinting of king scallops (Pecten maximus) reveals harvesting period and location. *The Science of the Total Environment, 697*, 134121. https://doi.org/10.1016/j.scitotenv.2019.134121

Nielsen, E. E., Cariani, A., Mac Aoidh, E., Maes, G. E., Milano, I., Ogden, R., … Carvalho, G. R. (2012). Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. *Nature Communications, 3*, 851. https://doi.org/10.1038/ncomms1845

NOAA. (2021). National oceanic and atmospheric administration: Cold and warm episodes by season. Retrieved from https://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php.

Ogden, R., & Linacre, A. (2015). Wildlife forensic science: A review of genetic geographic origin assignment. *Forensic Science International Genetics, 18*, 152–159. https://doi.org/10.1016/j.fsigen.2015.02.008

Ortea, I., & Gallardo, J. M. (2015). Investigation of production method, geographical origin and species authentication in commercially relevant shrimps using stable isotope ratio and/or multi-element analyses combined with chemometrics: An exploratory analysis. *Food Chemistry, 170*, 145–153. https://doi.org/10.1016/j.foodchem.2014.08.049

Özdilek, Ş. Y., Demir, N., & Gürkan, S. E. (2019). Assessment of pollution biomarker and stable isotope data in Mytilus galloprovincialis tissues. *Environmental Monitoring and Assessment, 191*(2), 60. https://doi.org/10.1007/s10661-019-7189-6

Page, H. M., & Lastra, M. (2003). Diet of intertidal bivalves in the Ra de Arosa (NW Spain): Evidence from stable C and N isotope analysis. *Marine Biology, 143*(3), 519–532. https://doi.org/10.1007/s00227-003-1102-z

Paulet, Y.-M. [Yves-Marie], Lorrain, A. [Anne], Richard, J., & Pouvreau, S. (2006). Experimental shift in diet δ13C: A potential tool for ecophysiological studies in marine bivalves. *Organic Geochemistry, 37*(10), 1359–1370. https://doi.org/10.1016/j.orggeochem.2006.01.008

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics, 18*(1), 293–320. https://doi.org/10.1146/annurev.es.18.110187.001453

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology, 83*(3), 703. https://doi.org/10.2307/3071875

Poulain, C., Gillikin, D. P., Thébault, J., Munaron, J. M., Bohn, M., Robert, R., et al. (2015). An evaluation of Mg/Ca, Sr/Ca, and Ba/Ca ratios as environmental proxies in aragonite bivalve shells. *Chemical Geology, 396*, 42–50. https://doi.org/10.1016/j.chemgeo.2014.12.019

Prendergast, A. L., Versteegh, E., & Schöne, B. R. B. R. (2017). New research on the development of high-resolution palaeoenvironmental proxies from geochemical properties of biogenic carbonates. *Palaeogeography, Palaeoclimatology, Palaeoecology, 484*, 1–6. https://doi.org/10.1016/j.palaeo.2017.05.032

Purchon, R. D. (1968). *The biology of the mollusca. International series of monographs in pure and applied biology Zoology* (Vol. 40). Oxford: Pergamon.

R Core Team. (2021). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/.

Ricardo, F., Génio, L., Costa Leal, M., Albuquerque, R. [Rui], Queiroga, H. [Henrique], Rosa, R., et al. (2015). Trace element fingerprinting of cockle (Cerastoderma edule) shells can reveal harvesting location in adjacent areas. *Scientific Reports, 5*, 11932. https://doi.org/10.1038/srep11932

Ricardo, F., Pimentel, T., Génio, L., & Calado, R. (2017). Spatio-temporal variability of trace elements fingerprints in cockle (Cerastoderma edule) shells and its relevance for tracing geographic origin. *Scientific Reports, 7*(1), 3475. https://doi.org/10.1038/s41598-017-03381-w

Riera, P. (2007). Trophic subsidies of Crassostrea gigas, Mytilus edulis and crepidula fornicata in the bay of mont saint michel (France): A δ13C and δ15N investigation. *Estuarine, Coastal and Shelf Science, 72*(1–2), 33–41. https://doi.org/10.1016/j.ecss.2006.10.002

Rumolo, P., Carannante, M., Gherardi, S., Tamburrino, S., Vallefuoco, M., Migliaccio, R., … Barra, M. (2017). Measuring stable carbon and nitrogen isotopes in Mytilus galloprovincialis to elucidate the sources of organic matter in three different nearshore marine environments. *Journal of Coastal Research, 335*, 1172–1181. https://doi.org/10.2112/JCOASTRES-D-16-00079.1

Sant'Ana, L. S., Ducatti, C., & Ramires, D. G. (2010). Seasonal variations in chemical composition and stable isotopes of farmed and wild Brazilian freshwater fish. *Food Chemistry, 122*(1), 74–77. https://doi.org/10.1016/j.foodchem.2010.02.016

Simenstad, C. A., & Wissmar, R. C. (1985). $\delta^{13}C$ evidence of the origins and fates of organic carbon in estuarine and near-shore food webs. *Marine Ecology Progress Series, 22*, 141–152. https://doi.org/10.3354/meps022141

Somes, C. J., Schmittner, A., Galbraith, E. D., Lehmann, M. F., Altabet, M. A., Montoya, J. P., … Eby, M. (2010). Simulating the global distribution of nitrogen isotopes in the ocean. *Global Biogeochemical Cycles, 24*(4). https://doi.org/10.1029/2009GB003767. n/a-n/a.

Steyn, H. S., Jr., & Ellis, S. M. (2009). Estimating an effect size in one-way multivariate analysis of variance (MANOVA). *Multivariate Behavioral Research, 44*(1), 106–129. https://doi.org/10.1080/00273170802620238

Strasser, C. A., Mullineaux, L. S., & Thorrold, S. R. (2008). Temperature and salinity effects on elemental uptake in the shells of larval and juvenile softshell clams Mya arenaria. *Marine Ecology Progress Series, 370*, 155–169. https://doi.org/10.3354/meps07658

Suh, Y. J., & Shin, K.-H. (2013). Size-related and seasonal diet of the manila clam (Ruditapes philippinarum), as determined using dual stable isotopes. *Estuarine, Coastal and Shelf Science, 135*, 94–105. https://doi.org/10.1016/j.ecss.2013.06.029

Thomas, A. C., Carr, M.-E., & Strub, P. T. (2001). Chlorophyll variability in eastern boundary currents. *Geophysical Research Letters, 28*(18), 3421–3424. https://doi.org/10.1029/2001GL013368

Titus, K., Mosher, J. A., & Williams, B. K. (1984). Chance-corrected classification for use in discriminant analysis: Ecological applications. *The American Midland Naturalist, 111*(1), 1–7. https://doi.org/10.2307/2425535

Trueman, C. N., & St John Glew, K. (2019). Chapter 6 - isotopic tracking of marine animal movement. In K. A. Hobson, & L. I. Wassenaar (Eds.), *Tracking animal migration with stable isotopes* (2nd ed., pp. 137–172). Academic Press. https://doi.org/10.1016/B978-0-12-814723-8.00006-4.

Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution, 193*, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010

Westfall, K. M., & Gardner, J. P. A. (2013). Interlineage Mytilus galloprovincialis Lmk. 1819 hybridization yields inconsistent genetic outcomes in the Southern hemisphere. *Biological Invasions, 15*(7), 1493–1506. https://doi.org/10.1007/s10530-012-0385-8

Zardi, G. I., McQuaid, C. D., Jacinto, R., Lourenço, C. R., Serrão, E. A., & Nicastro, K. R. (2018). Re-assessing the origins of the invasive mussel Mytilus galloprovincialis in southern Africa. *Marine and Freshwater Research, 69*(4), 607. https://doi.org/10.1071/MF17132

Zbawicka, M. [Małgorzata], Trucco, M. I., & Wenne, R. (2018). Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels: Hybridization with invasive European Mytilus galloprovincialis. *Genetics Selection Evolution : GSE, 50*(1), 5. https://doi.org/10.1186/s12711-018-0376-z

Zhao, X., Liu, Y. [Yu], Wang, G., Tao, W., Lou, Y., Li, N., et al. (2019). Tracing the geographical origins of Yesso scallop (Patinopecten yessoensis) by using compound-specific isotope analysis: An approach for overcoming the seasonal effect. *Food Control, 102*, 38–45. https://doi.org/10.1016/j.foodcont.2019.03.016

Zhao, L., Milano, S., Tanaka, K., Liang, J., Deng, Y., Yang, F., et al. (2020). Trace elemental alterations of bivalve shells following transgenerational exposure to ocean acidification: Implications for geographical traceability and environmental reconstruction. *The Science of the Total Environment, 705*, 135501. https://doi.org/10.1016/j.scitotenv.2019.135501