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Current trends in the traceability of geographic origin and detection of species-mislabeling in marine bivalves

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ABSTRACT

Marine bivalves are increasingly consumed worldwide, with their complex supply chain being particularly prone to fraud. This scenario drives economic losses and is a threat to public health, with multiple recent food worries driving consumers to demand more transparency and information on the seafood they buy. To increase consumers confidence in bivalves and enforce current legislation, robust tools are needed to fight species mislabeling and confirm the place of origin of bivalves being traded.

The present study provides a critical overview based on a databases search, over the traceability of geographic origin and detection of species-mislabeling in marine bivalves, summarizing the tools currently available to confirm claims on these topics along the supply chain. We also identify current trends on the use of tools, pinpoint which countries contribute to advance the state of the art on these topics, and highlight the bivalve groups/species being more commonly surveyed.

The most used tools to expose species mislabeling in marine bivalves are DNA and fatty acid analysis, while elemental analysis is the most commonly employed approach to confirm their geographic origin. Stable and unstable isotope analysis, as well as metabolomics, are also starting to be increasingly used to verify species authenticity and provenance in marine bivalves. Further studies are still needed to identify annual/seasonal variations and determine if these can be a constraint for the optimization of protocols to fight fraudulent practices. The implementation of an open global database to allow realtime data comparison will be paramount to advance the state of the art.

1. Introduction

Bivalves (e.g., clams, mussels, oysters, and scallops) represent an important contribution to human nutrition and health, and are also appreciated for cultural and gastronomic reasons (Golden et al., 2021). Their worldwide consumption per capita in 2020 was estimated to be 1.93 kg, with China being the nation that most consumed bivalves (with Hong Kong recording a 13.71 kg bivalve consumption per capita), followed by South Korea (at 9.74 kg per capita), Japan (at 5.65 kg per capita), and several European Union countries (e.g. Spain at 9.16 kg per capita, Italy at 5.37 kg per capita and France at 5.35 kg per capita) (EUMOFA, 2021; FAO, 2022a). Due to the high demand for bivalves, its worldwide production has grown significantly in recent decades, having increased by nearly 70% in the last 20 years (FAO, 2022b; 2022c). Aquaculture production emerged in this period with an increase of about nearly 90%, while wild catch declined by more than 25% (FAO, 2022b; 2022c).

The imports and exports of bivalves also increased significantly until 2019, although in 2020 these values dropped by nearly 10% compared to the previous year due to the COVID-19 pandemic (FAO, 2022d). In 2019, export volumes reached 950 thousand tonnes and imports achieved 872 thousand tonnes, representing a total value of USD 4.2 billion

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and USD 3.8 billion, respectively (FAO, 2022e). The top importing countries of bivalves were China, Japan, the USA, France, and South Korea, with nearly a ten of countries worldwide representing about 80% of all bivalves imports (FAO, 2022e). China commonly imports scallops from Japan and exports large numbers of bivalves to Japan, the USA, South Korea, and Thailand (FAO, 2022e). The USA mainly imports mussels from Canada and Chile, while France imports them from Chile, Spain, and the Netherlands (FAO, 2022e). Spain mostly imports mussels from Chile, Scallops from France, and clams from Vietnam, while it exports mussels to Italy and Portugal (FAO, 2022e). Portugal, in turn, mainly imports clams from Vietnam, while Belgium imports mussels from the Netherlands (Fig. 1) (FAO, 2022e).

Overall, bivalves supply chains are global, being highly complex and often blurry, thus increasingly vulnerable to fraud (El Sheikha & Montet, 2016; Fox et al., 2018; Leal et al., 2015). This fraud frequently leads to economic loss and potential risks to public health due to mislabeled or undeclared products that may contain toxins, human pathogens or other pollutants, leading to allergic reactions, foodborne illness or other negative effects on human health (Hassoun et al., 2020; Jennings et al., 2016). The perishable nature of seafood makes it of higher concern in terms of food safety than other foods (Leal et al., 2015). As a result of numerous food fraud controversy in recent years, namely related with the mislabeling of seafood (Colihueque et al., 2020; Giusti et al., 2020; Lawrence et al., 2022; Parrondo et al., 2021; Spielmann et al., 2018; Wen et al., 2018), consumers have become more concerned on the origin and safety of the food products they acquire (Hassoun et al., 2020; H. Ye et al., 2023). Consumers increasingly want more transparent and complete information (from farm to fork) on the product they buy, namely the place of production/harvesting, as well as when and how was their production/harvesting performed (Hassoun et al., 2020).

2. Traceability of marine bivalves - application and relevance

The traceability of marine bivalves is increasingly becoming a requirement for the sustainable management and conservation of these important marine organisms and food products, as well as to safeguard public health and provide consumers with more reliable information on the products they acquire (El Sheikha & Montet, 2016; Leal et al., 2015). The concept of traceability is defined as "the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing, and distribution" (EC, 2002).

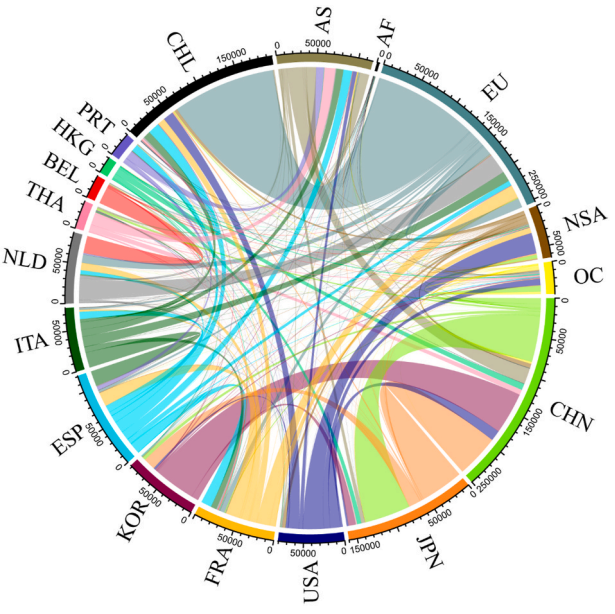

Fig. 1. Chord diagram representing the global trade network of bivalves in 2020 considering the twelve most relevant importers and Chile. China (CHN), Japan (JPN), United States of America (USA), France (FRA), South Korea (KOR), Spain (ESP), Italy (ITA), Netherlands (NLD), Thailand (THA), Belgium (BEL), Hong Kong (HKG), Portugal (PRT), Chile (CHL), Asia (AS), Europe (EU), Oceania (OC), Africa (AF) and North, Central and South America (NSA) (Source: FAO).

Traceability ensures product quality, providing a value guarantee that attends consumer demand preferring high quality and environmentally friendly products (Gopi et al., 2019; Pieniak et al., 2013). It is also extremely important to minimize food safety risks, as authorities can trace the origin of a contaminated product, apply a contingency plan and ascertain responsibilities quickly and effectively (Fox et al., 2018; Leal et al., 2015; Power & Cozzolino, 2020). Traceability can also certify the legal and sustainable origin of products and minimize illegal and unreported captures, thus fostering sustainable fisheries management (Helyar et al., 2014; Leal et al., 2015). Finally, another problem that can be avoided is fraudulent labeling, either by shifting species name or by its provenance (El Sheikha & Montet, 2016).

The European Union has enhanced seafood traceability regulations and requirements when contrasted with other markets worldwide (Charlebois et al., 2014; Hall & Johnson-Hall, 2021; Lindley, 2022). In 2000, the European Regulation (EC) No.104/2000 was developed, which requires an "appropriate marking or labeling indicating: (a) the commercial designation of the species; (b) the production method (caught at sea or in inland waters or farmed); (c) the catch area" (EC, 2000). In the following year, this regulation was updated and the scientific name of the species on the label was also required (EC, 2001). In 2002, the European Union defined the term traceability for the first time and established regulatory requirements in EC No.178/2002 (EC, 2002). Later, the European Union developed specific requirements for traceability, which requires that "all lots of fisheries and aquaculture products shall be traceable at all stages of production, processing, and distribution, from catching or harvesting to retail stage" (EC, 2009). In 2013, European Regulation No.1379/2013 was developed, which "contributes to the traceability of fishery products and to clear and comprehensive information for consumers", requiring, in addition to the information already required on labels, the name of the fishing gear used to harvest fishery products (EU, 2013).

The overall success of these legal measures depends, among other things, on the development of reliable traceability tools to confirm claims on bivalve labels about species name and geographic origin, to ensure their safety for human consumption (Leal et al., 2015; Ricardo et al., 2021). The present work provides a critical overview on the scientific literature produced since the year 2000 on the tools available to trace the geographic origin of bivalves and detect species-mislabeling on this commercially important group of seafood.

3. Materials and methods

A literature search was performed in January 2023on the databases Thomson Reuters Web of Science (Core Collection) (Topic) and Scopus (Article title, Abstract, Keywords) to identify relevant studies on the traceability of geographic origin and detection of species-mislabeling in bivalves using the following keywords: (traceability OR provenance OR fingerprint OR authentication OR "geographic origin" OR certification OR mislabeling OR "species identification") AND (bivalve OR clam OR cockle OR mussel OR oyster OR scallop). Original studies were eligible if they directly addressed the traceability of marine or brackish water bivalves and fulfilled at least one of the following criteria: addressed the geographic origin of bivalves and/or species-mislabeling of bivalves (Fig. 2). Briefly, a total of 1598 relevant studies were retrieved from the two databases. Subsequently, 632 articles were excluded as they were duplicates or were not original studies. The remaining 966 articles were fully screened, with 825 being excluded for not fulfilling the inclusion criteria detailed above. Consequently, the remaining 141 articles were considered relevant and selected for further analysis (Supplementary

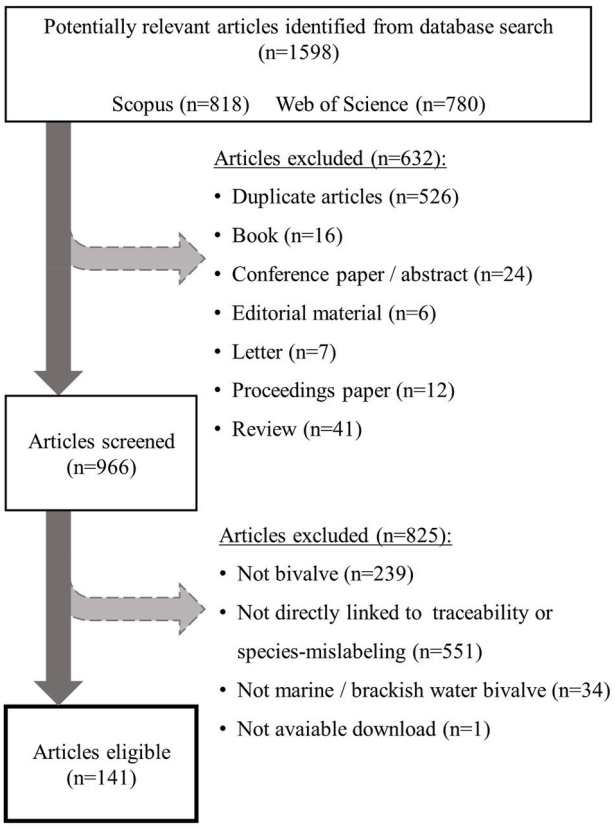

Fig. 2. Flow chart of the screening and selection process of peer reviewed scientific publications on the traceability of geographic origin and detection of species-mislabeling in bivalves.

Table S1). The following data were accessed: authors, year of publication, country, tool employed, resolution (maximum and minimum distance between locations), bivalve species, biological matrix surveyed, type of bivalve processing, and study objective. All species names were verified according to WoRMS Editorial Board (2022), to assure that only currently valid scientific names were used. Over the "Results and Discussion" section, only the publications that better illustrate each potential application/tool are cited, with all others being made available in the supplementary material (Supplementary Table S1).

4. Results and discussion

4.1. General framework

Since the year 2000, the interest on traceability of geographic origin and detection of species-mislabeling has increased resulting in a growing number of scientific articles on this topic (Fig. 3), with a total of 141 articles published. The common goal of these studies was to investigate the geographical origin and species-mislabeling of bivalves, with these topics accounting for 54% and 46% (respectively) of the total number of publications selected for the present study. To pursue these goals, a total of six different tools were used, namely: DNA, fatty acids, metabolomics, elemental, and stable and unstable isotope analyses (Fig. 3). Several countries have investigated on the traceability of geographic origin of bivalves and detection of species-mislabeling, with Spain, China, Portugal, and Italy being the most prolific. Globally, a resolution range between 0.7 and 20000 km was achieved when addressing the traceability of geographic origin. Of all possible species, 138 species/genera were surveyed, all of these being listed in FAO International Standard Statistical Classification for Aquatic Animals and Plants (ISSCAAP) groups, namely: clams, cockles, and arkshells (66 species), mussels (13 species), oysters (35 species), and scallops and pectens (24 species). Soft tissues, adductor muscle, shell, foot, mantle, gills, digestive glands, pearl, and periostracum were the biological matrices employed in the studies, with the first three (soft tissues, shell, and adductor muscle) accounting for 70% of all publications analysed. Finally, 14 types of bivalve sample processing techniques were identified, with these being grouped into dried (air-dried and dried), cooked (boiled, cooked, and pre-cooked), canned, fresh, frozen, frozen cooked (frozen cooked and frozen pre-cooked), and others (marinated, n.s., old, and processed), with 75% of selected publications referring to "fresh" bivalve samples.

4.2. Different approaches to bivalves' traceability of geographic origin and detection of species-mislabeling - tools

4.2.1. DNA analyses

Some analytical methods used for species identification and food authentication are based on DNA analyses (Mafra et al., 2008). DNA analysis has been the most employed method due to its high stability,

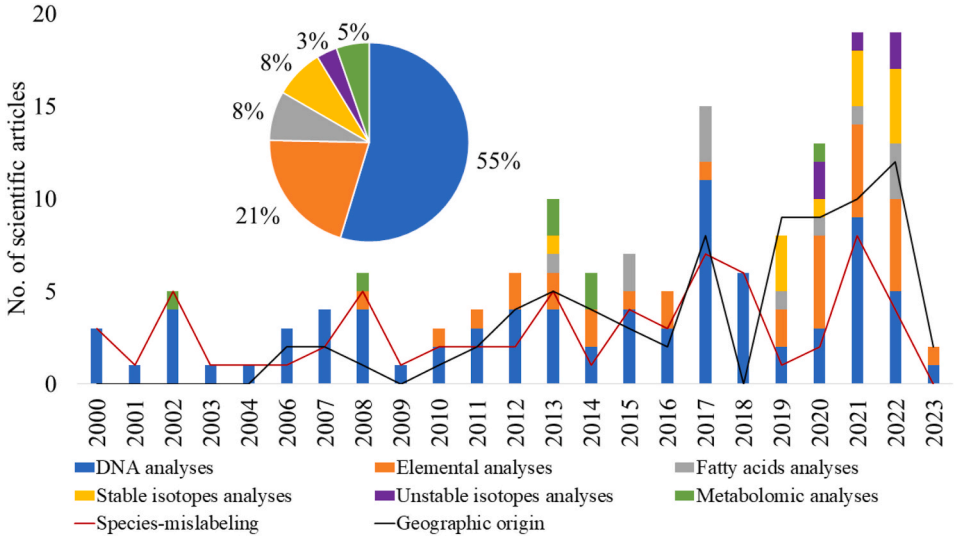

Fig. 3. Number of peer reviewed publications in international journals on the traceability of geographic origin and detection of species-mislabeling in bivalves per type of tool employed and per year (bar graph); proportion of peer reviewed publications in international journals on these research topics per type of tool used (pie chart).

and its presence in most biological tissues (Mafra et al., 2008). Most DNA-based methods consist of specific amplification of one or more DNA fragments by polymerase chain reaction (PCR) (Mafra et al., 2008; Pirondini et al., 2010). The specific amplification of a fragment followed by electrophoresis in agarose gel to verify the size of the fragment is a simple, fast, and highly sensitive technique (Mafra et al., 2008). Fragment confirmation and analysis can also be performed by simultaneously amplifying two or more fragments with different primer pairs (Multiplex PCR), Restriction Fragment Length Polymorphism (PCR-RFLP), Restriction-Site Associated DNA Sequencing (RAD-seq), real-time PCR and DNA barcoding (del Rio-Lavín et al., 2021, 2022; Fernàndez-Tajes et al., 2012; Gense et al., 2021; Klapper & Schröder, 2021; Parrondo et al., 2021; Y. Y. Ye et al., 2012).

DNA analyses have been the most used tool for species identification and the second most used to discriminate the geographic origin of bivalves (Fig. 3) (Colihueque et al., 2020; del Rio-Lavín, Weber, et al., 2022; Fernández-Pérez et al., 2018; Fernàndez-Tajes et al., 2012; Freire et al., 2008; Klapper & Schröder, 2021; Larraín et al., 2019; Mazón-Suástegui et al., 2016; Parrondo et al., 2021; Velez-Zuazo et al., 2021; Wen et al., 2018). This tool appears to be relatively rapid and cost-effective, however, DNA is susceptible to degradation (Fig. 4) (Table 1) (Gopi et al., 2019; Leal et al., 2015). Most DNA analyses for bivalves' traceability and species identification consist of amplification of the mitochondrial cytochrome oxidase subunit I (*COI*) gene and 16S ribosomal RNA (*16S rDNA*) gene through the techniques referred above. The use of mitochondrial genes for the identification of some families of marine bivalves (Mytilidae and Veneridae) is not advised, instead, ribosomal genes should be used. While in most animals mitochondrial DNA is exclusively inherited from the mother, this does not happen in these two bivalve families (Birky, 2001). In Mytilidae and Veneridae there is a deviation from this norm termed as doubly uniparental inheritance (DUI), with bivalves being characterized by distinct gender-associated mitochondrial DNA that is inherited from its mother or father (Breton et al., 2007).

In recent decades, several studies seek to identify species of marine bivalves and develop more accurate methodologies for each species. Fernández-Pérez et al. (2018) developed a methodology for the correct identifications of four species of clams (*Donax semistriatus, D. trunculus, D. variegatus,* and *D. vittatus*) originating from the Iberian Peninsula, using multiplex PCR amplification of the *5S rDNA* and the *ITS*. Similarly, Freire et al. (2008) employed PCR-RFLP using *ITS1* to distinguish two species of clams (*Ensis arcuatus*, and *Ensis siliqua*) from the Spanish coast. In turn, Mazón-Suástegui et al. (2016) evidenced that PCR amplification of *28S rDNA* was able to differentiate nine commercially important oysters (*Magallana gigas*, *M. sikamea*, *Crassostrea virginica*, *C. rhizophorae*, *C. corteziensis*, *C. columbiensis*, *Saccostrea palmula*, *Striostrea prismatica*, and *Ostrea chilensis*). Larraín et al. (2019) were also able to differentiate four species of mussels (*Mytilus edulis*, *Mytilus galloprovincialis*, *M. chilensis*, and *M. trossulus*), using PCR-RFLP screening of *Me15-16*, *ITS*, *mac-1*, *16S rDNA* and *COI* markers. In a first approach, the authors compared the species using a marker alone and in a second approach they used the five markers together, highlighting the multi-locus approach that clearly identified the four species.

Along with traditional species identification, there has been an increase in studies that seek to prove the correct labeling of marine bivalves. For example, in the Asian market, dried oysters, clams, and mussels are widely consumed; however, there is often no indication of the species traded. To assess which species were present in this trade, Wen et al. (2018) used PCR amplification of the *COI* gene. The study showed that 81% of the oysters being traded dry belonged to the species *Magallana angulata* and 19% to *M. gigas*; 58% of the clams being traded belonged to *Mactra chinensis* and 42% to *Ruditapes philippinarum*; while 100% of the mussels being traded were *M. galloprovincialis*.

Similarly, Colihueque et al. (2020) used the PCR-RFLP method based on the analysis of the *18S rDNA* gene, in order to authenticate the specimens of 6 commercial brands of frozen *M. chilensis* mussel being sold in Chile. Three of the brands proved to be well identified, while 13–50% of individuals from the other three brands were mislabeled (these were actually specimens of *Aulacomya atra*). Parrondo et al. (2021) evaluated the mislabeling of scallops with a commercial interest in Galicia (Spain), using PCR amplification of the mitochondrial *COI* gene and the *16S rDNA* gene in soft tissues. Scallops from supermarkets, small fish shops, and gourmet shops were analysed and divided into fresh, frozen, and canned products. DNA analysis showed that 25% of the fresh scallops, 50% of frozen, and 100% of canned scallops were mislabeled and did not match the species being referred in products labels. In addition to these commercial spaces, scallops were also sampled in restaurants where an incidence of 100% species mislabeling was detected. Another study performed in Germany also, assessed the accuracy of labeling on scallops products (Klapper & Schröder, 2021). The authors analysed three different species of scallops (*Pecten* spp., *Placopecten magellanicus*, and *Mizuhopecten yessoensis*), fresh, frozen, and canned from supermarkets, fishmongers, and restaurants. By using multiplex real-time PCR screening *16S rDNA*, *COI*, and *cytochrome b*

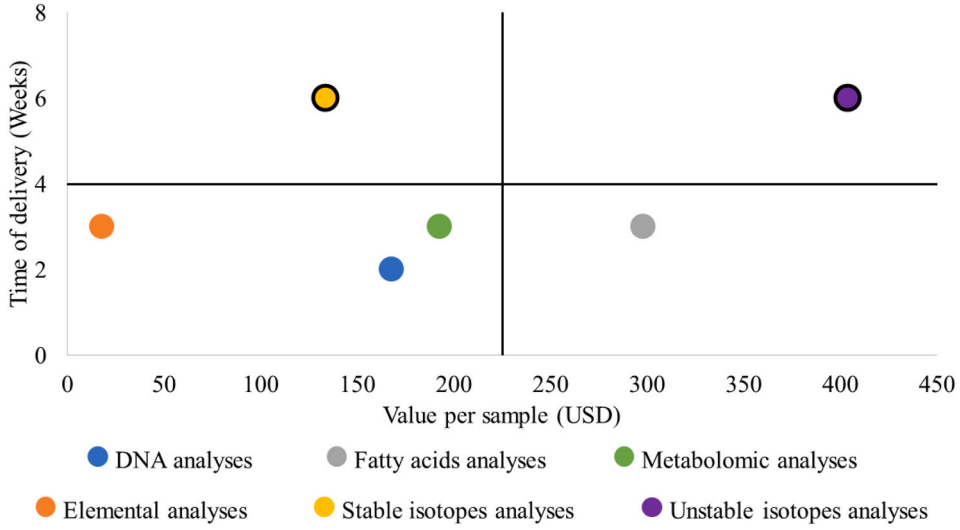

Fig. 4. Quadrant chart evaluating the average time for processing samples and delivering results (in weeks) and associated average cost per sample (in Euros) for each tool. Black border marker - value per isotope per sample. The average time elapsing since the reception of samples to the delivery of results, as well as average costs per sample, were calculated using proposals received from several laboratories worldwide performing the different techniques addressed in this study.

Table 1
Advantages and limitations of current tools employed in the traceability of geographic origin and detection of species-mislabeling in marine bivalves. * Average time for delivery of results by laboratories. The average time for delivery of results and average costs per sample (one single specimen) were calculated using proposals received from several laboratories worldwide performing the different techniques addressed in this study.

	Advantages	Limitations
DNA analyses	• Used to distinguish between geographic origins and species-mislabeling • Cost-effective (±168USD per sample) • Rapid (2 weeks*) • Low temporal shift • Can be immediately compare inter laboratory	• Cannot differentiate close geographical areas • DNA is susceptible to degradation
Fatty acids analyses	• Used to distinguish between geographic origins and species-mislabeling • Cost-effective (±298USD per sample)	• Slower than other methods (3 weeks*) • Samples require extensive preparation • Cannot be applied in processed bivalves • The fatty acids compositions changes over time • Lipids are susceptible to oxidation
Metabolomic analyses	• Used to distinguish between geographic origins and species-mislabeling • Cost-effective (±193USD per sample)	• Slower than other methods (3 weeks*) • Samples require extensive preparation
Elemental analyses	• Used to distinguish between geographic origins and time of harvest • Cost-effective (±18USD per sample) • Simple methodology • Not susceptible to degradation	• Cannot be applied in soft tissues of processed bivalves • Slower than other methods (3 weeks*) • Hight temporal shift
Stable isotope analysis	• Used to distinguish between geographic origins and species-mislabeling • Cost-effective (±134USD per isotope per sample) • Simple methodology	• Slower than other methods (6 weeks*) • Hight temporal shift
Unstable isotopes analyses	• Used to distinguish between geographic origins	• High cost (±404USD per isotope per sample) • Slower than other methods (6 weeks*) • Relatively complex methodology • Hight temporal shift

genes, the authors revealed that only 52% of the scallops were correctly labeled. Supermarket scallops showed an average incidence of mislabeling around 24%, while in fishmongers and restaurants 80% and 100% (respectively) of the scallops surveyed were mislabeled.

DNA analyses have also been used to trace the geographical origin of bivalves. Del Rio-Lavín et al. (2022) aimed to develop and evaluate a tool based on the Single Nucleotide Polymorphism (SNP) to assign samples to their specific geographic origin. To accomplish this, the mantle of mussel *M. galloprovincialis* from 9 different aquaculture locations, distributed across the Atlantic Ocean (Portugal and Spain), the Mediterranean Sea (Spain and Tunisia), and the South-eastern Pacific Ocean (Chile) were investigated, obtaining SNP markers by RAD-sequencing. Mussels from the Atlantic Ocean showed high genetic differentiation compared to those from the Mediterranean Sea and the South-eastern Pacific Ocean, with the correct allocation to the place of origin ranging between 90 and 100%. While the authors demonstrated that it was possible to distinguish the geographic origin of mussels through DNA analyses, they also emphasized that it is not possible to differentiate samples from different locations within the same geographic area, indicating that in these cases a multidisciplinary approach would most likely be required. Similarly Velez-Zuazo et al. (2021) used SNP markers obtained from next-generation RAD-sequencing of the adductor muscle of the scallop *Argopecten purpuratus* from 5 natural banks distributed between Peru and Chile. They were also unable to differentiate scallops from natural banks within Peru, but successfully discriminated specimens originating from Peru and Chile.

Fernàndez-Tajes et al. (2012) attempted to differentiate clams harvested from Portugal, Spain, and Ireland and verify the accuracy of labeling of cans detailing the geographical origin of razor clams (genus *Ensis*). For this purpose, the foot of *E. siliqua* collected in 3 locations of each country of origin was used for PCR using a microsatellite marker. The authors were able to differentiate the clams from Ireland and the Iberian Peninsula, with only 1 of the 6 canned samples corresponded to the geographical origin indicated on the label - Galicia.

4.2.2. Fatty acids analyses

Fatty acids (FA) are the major constituent of triglycerides (TAG) and polar lipids (PL) (Bergé & Barnathan, 2005). They are composed by an alkyl chain with a terminal carboxylic acid group (-COOH), that can be esterified to a glycerol backbone in the case of TAG and PL (Arts et al., 2009). Fatty acyl chains can have different chain length, ranging from 4 to 36 carbons, and can have some doubles bonds (n = 0 to 6) (Bergé & Barnathan, 2005). The fatty acid profile in bivalves can be modulated by several intrinsic (e.g., age, sex, reproductive cycle, and phylogeny) and extrinsic factors (e.g. diet, temperature, depth, and salinity) (Zhukova, 2019). The latter make FA strong biomarkers of environmental conditions, being particularly useful for the traceability of their geographical origin (Leal et al., 2015). The diet available for bivalves differs with the ecosystem where they live, thus affecting the composition of their FA profile (Zhukova, 2019). This dietary shaping of fatty acid profiles can be a caveat when comparing samples from the same location but collected in different seasons; however, such bias caused by seasonality associated with diet can be minimized through the use of the adductor muscle as the biological matrix to be screened (Grahl-Nielsen et al., 2010; Leal et al., 2015). The adductor muscle is mostly composed by polar lipids, which are less prone to changes due to feeding regimes (Grahl-Nielsen et al., 2010; Leal et al., 2015).

Fatty acid analyses typically use methods of chromatography or spectroscopy, such as Gas Chromatography-Mass Spectrometry (GC-MS), Gas Chromatography with Flame Ionization Detection (GC-FID), and Liquid Chromatography with tandem Mass Spectrometry (LC-MS-MS). This tool is relatively low-cost, although its performance is slower than others available for this task due to the amount of time required for sample preparation (Fig. 4) (Gopi et al., 2019; Leal et al., 2015). In addition, lipids are susceptible to oxidation, which precludes the use of processed products (Table 1) (Gopi et al., 2019; Leal et al., 2015). This tool has already been demonstrated to be reliable when aiming to perform geographic origin discrimination of bivalves (Costa et al., 2017; Fonseca et al., 2022; Go et al., 2022; Liu et al., 2022; Mamede et al., 2020; Ricardo et al., 2015, 2017; Xu et al., 2015; Zhang et al., 2019), despite not being the most used one for this purpose (Fig. 3).

Fonseca et al. (2022) employed FA analyses to determine the geographic origin of *Scrobicularia plana* clam within the Tagus estuary (Portugal), where its capture is prohibited due to high concentrations of lead (IPMA, 2021). In this study, soft tissues from fresh clams captured within 3 locations were used and the authors detected 19 FAs in these samples using GC-FID. Canonical Analysis of Principal coordinates (CAP) correctly allocated with 100% certainty the harvesting locations of these clams. Such results demonstrate that the FA profile presents great potential as a tracer of geographic origin of bivalves, even when screening specimens at small spatial scales.

Shifts in the FA profile over lifetime can be a concern, even with the use of the adductor muscle, which has lower turnover rates promoted by short-term trophic and environmental variability (Zhukova, 2019). The

lipid composition of the adductor muscle is mainly related to environmental conditions, rather than short-term shaping of dietary regimes, predicting that changes in the FA profile will only likely occur after long periods of time (Grahl-Nielsen et al., 2010). Ricardo et al. (2017) studied the annual variation of the FA profile of fresh cockles *Cerastoderma edule* from the Ria de Aveiro (Aveiro, Portugal). GC-MS analysis revealed a profile of 21 FA in the adductor muscle and revealed significant differences between harvesting years. This study supported that FA analysis can be used to discriminate geographic origin at variable spatial scales, although, caution is necessary when aiming to compared data over different years due to annual variability.

Another recent study demonstrated that FA analyses hold the potential to combat the illegal, unreported, and unregulated (IUU) capture of bivalves, which endangers important habitats and threatens public health (Mamede et al., 2020). The authors used FA analysis of the adductor muscle of Manila clams *R. philippinarum* to verify whether clams suspected from being illegally harvested from the Tagus estuary (Lisbon, Portugal) were indeed harvested from this area where its capture is forbidden due to historical contamination with metals. For this purpose, the relative abundance of 26 FA present in the adductor muscle of these clams was determined using GC-MS. Additional clams were collected from Ria de Vigo (Galicia, Spain), Ria de Aveiro, and the Tagus estuary (Portugal), ecosystems that are important suppliers of this species to the Portuguese market of live bivalves. A model built with a training dataset was created by CAP, which revealed a high performance and correctly allocated 100% of these clams with unknown origin, showing that about nearly 75% of them were indeed illegally harvested from the Tagus estuary.

4.2.3. Metabolomic analyses

Metabolomics allows the mass screening of several metabolites in cells, tissues, or organisms (the metabolome), such as small peptides, oligonucleotides, sugars, organic acids, ketones, aldehydes, amino acids, lipids, steroids, alkaloids, and xenobiotics (Alfaro & Young, 2018; Cubero-Leon et al., 2014). Metabolomic analyses can be classified as targeted, focusing on a specific group of metabolites, which in most studies requires the identification and classification of all metabolites within the studied group, or non-targeted, which focuses on the detection (identification and quantification) of all possible metabolites to obtain a fingerprint (Patti et al., 2012; Ramautar et al., 2006). Depending on the purpose of the analyses, this tool can also be classified as informative, discriminative, or predictive (Cevallos-Cevallos et al., 2009). In informative metabolomics, metabolites are identified and quantified to obtain information. Discriminative analysis aims to find differences between samples, while predictive analysis allows to create statistical models with the metabolomic profile to predict class associations (Cevallos-Cevallos et al., 2009).

The profile of metabolites differs with genotype and environmental conditions shaping the growth of the organisms being screened (Patti et al., 2012), thus providing reliable information on their geographic origin through discriminative and predictive metabolic analyses (Cevallos-Cevallos et al., 2009). Indeed, metabolomic studies have already been carried out to trace the geographic origin of bivalves (Fig. 3) (Aru et al., 2020; Ielmini et al., 2014; López et al., 2002; Ratel et al., 2008; Rocha et al., 2013; Rochfort et al., 2013; Stephan et al., 2014), using Solid Phase Microextraction - Mass Spectrometry (SPME-MS), Comprehensive two-dimensional Gas Chromatography coupled to Time-of-Flight Mass Spectrometry (GC × GC–ToFMS) and nuclear magnetic resonance spectroscopy (Nuclear Magnetic Resonance (NMR). While cost-effective, these analyses are slower to performed when compared to other tools available due to the complexity of sample preparation and the analysis of results (Fig. 4) (Table 1).

Metabolomic analyzes were used to discriminate mussels (*M. edulis*) and clams (*R. philippinarum*) purchased in Denmark and Italy (Aru et al., 2020). Soft tissue was analysed by NMR for determination of mytilitol and through PCA it was possible to verify that this metabolite is species-specific and differs with geographic origin.

Alternatively, Rochfort et al. (2013), investigated the potential of metabolomic analyses to differentiate not only the place of origin but also the species of mussel samples. The authors started by analyzing the metabolites present in the soft tissues of *M. galloprovincialis* from Australia and *Perna canalicus* from New Zealand using 1H NMR. A PCA with 65% total variation completely separated the two species. In the second part of the study, metabolites of *M. galloprovincialis* from two aquaculture facilities in Port Phillip Bay (Australia), about 18 km apart, were analysed and a PCA partially separated mussel specimens originating from the two locations.

For a stronger assessment of the potential of metabolomics to discriminate bivalve species, Rocha et al. (2013) analysed two species of clams (*Ruditapes decussatus* and *R. philippinarum*) present in Ria de Aveiro using GC × GC–ToFMS. Nearly 200 volatile compounds were recorded, 63 of which successfully discriminated the two species, thus evidencing the potential of this approach to expose species-mislabeling, even of closely related species (same genus).

4.2.4. Elemental analyses

Bivalves have been recognized as bioindicators of environmental quality in aquatic systems (Cajaraville et al., 2000). These filter-feeding species have a natural tendency to accumulate metals in their soft tissues and shells as they grow (Fortunato, 2015; Labrecque et al., 2004), being influenced by intrinsic species-specific features, as well as by abiotic conditions, namely salinity gradients, temperature, tidal variations and water turbidity (Eggleton & Thomas, 2004). The accumulation of metals results in elemental fingerprints (EF) that closely reflect the environmental conditions that bivalves have experienced until their time of harvest (Fortunato, 2015; Labrecque et al., 2004).

Elemental analyses using the EF of bivalves have been the most used tool to trace their geographic origin (Fig. 3) (Bennion et al., 2021; Broadaway & Hannigan, 2012; Costas-Rodríguez et al., 2010; Iguchi et al., 2014; Mamede et al., 2022; Morrison et al., 2019; Mouchi et al., 2021; Ricardo et al., 2020, 2022; Sorte et al., 2013). It generally employs the Inductively Coupled Plasma (ICP) method, a powerful chemical analysis approach that can be used to identify trace and major concentrations of nearly all elements in a sample (Zoorob et al., 1998). This method branches into ICP - Mass Spectrometry (ICP-MS), ICP - Atomic Emission Spectroscopy (ICP-AES), also known as ICP - Optical Emission Spectroscopy (ICP-OES), and Laser Ablation - Inductively Plasma Coupled - Mass Spectrometry (LA -ICP-MS). These methods can be used both in soft and hard calcified tissues samples of bivalves, requiring previous sample digestion, except for the latter where only the shell is used (Gopi et al., 2019). Elemental analyses are a relatively low-cost, simple methodology, which is not susceptible to element degradation post-harvesting (Fig. 4) (Table 1) (Gopi et al., 2019; Leal et al., 2015).

Sorte et al. (2013) used the elemental fingerprint of the shell of the mussel *M. edulis* to determine their harvesting location. For this purpose, mussels were collected at 7 locations between northern Main and Cape Code (Massachusetts, USA), separated between them from 50 up to 500 km. The shells were analysed by LA-ICP-MS and 8 elements were used to tell apart locations through the Linear Discriminant Function, which revealed nearly 70% of correct allocation to the place of origin of mussels. Aiming to achieve a similar goal, Mamede et al. (2022) determined the EF of the shell of clams using ICP-MS to discriminate the origin of *R. decussatus* collected over 8 locations and *R. philippinarum* in 7 locations along the Atlantic NW, W, and SW Iberian coast, distancing between them in17 and 560 km. The concentration of 9 elements present in the shell of these clams was used to perform a Random Forest classification that correctly allocated 96% and 98% of *R. decussatus* and *R. philippinarum*, respectively, to their place of origin.

In a more comprehensive study, Ricardo et al. (2022) evaluates the accuracy of the EF in the allocation to the place of origin of the cockle *C. edule* shell on three different spatial scales: regional spatial scale (Galicia coast), national spatial scale (Portuguese coast) and

international spatial scale (Northeast Atlantic cost), with the locations separated between 9 and 209 km, 47–400 km and 185–3350 km, respectively. The EF of the cockle shell was analysed by the ICP-MS, and through 5 elements the Random Forest classification was able to correctly allocate 97% of the cockles on the regional spatial scale, 99% on the national and 100% on the international spatial scale to the place of origin.

Since the accumulation of metals in bivalves is influenced by abiotic conditions, EF may shift with changes in the environment. Bennion et al. (2021) investigated the stability/variability of EF in the mussel *M. edulis* EF from Killary Fjord (Ireland) on 5 different occasions, for three years, while also determining the best matrix or combination of matrices to use when aiming to trace the place of origin of these bivalves. The concentrations of 18 elements from four matrices (periostracum, foot, unclean shell, and clean shell) were determined using ICP-MS and employed to test if mussels were correctly assigned to their respective time of harvest applying a Random Forest classification. The percentage of correct allocation to the time of harvest was always equal to or greater than 80% with a maximum of 96% when using the combined EF of the clean shell and periostracum, as well as clean shell and foot. This approach demonstrated that EF analyses can not only identify the geographic origin of bivalves but also the date of harvest, considering that further studies are needed in different species to test the accuracy of this tool concerning temporal variation.

4.2.5. Stable isotope analyses

Isotopes are atoms of one element, which have the same number of protons but different numbers of neutrons (Camin et al., 2016). Isotopes are classified as stable, which are not subject to any radioactive disintegration, or as unstable (Jafari et al., 2020; Koletzko et al., 1997), which will be discussed further below. Primary producers have a distinct isotopic fingerprint, and when consumed by bivalves, this fingerprint is assimilated into their tissues through a process known as fractionation (Ehleringer et al., 1986). Consequently, such as EF, stable isotopes accumulate in the tissues and shells of bivalves.

Stable isotope analyses have been used for more than a decade to trace the geographic origin of bivalves (Fig. 3) (Bajnóczi et al., 2013; Bianchini et al., 2021; del Rio-Lavín, Weber, et al., 2022; Kang et al., 2022; Matos et al., 2021; Milano et al., 2020; Zhang, et al., 2019a, 2019b; Zhao, Liu, et al., 2019), using carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), hydrogen ($\delta^{2}H$), and sulfur ($\delta^{34}S$) isotopes. The variation of carbon and nitrogen isotopes is strongly related to diet, with the first indicating the source of nutrients and the second indicating the trophic level of an organism in the food chain (Camin et al., 2016; Gopi et al., 2019; Vinci et al., 2013). Oxygen and hydrogen concentration is directly related to the availability of these isotopes in seawater, being affected by evaporation, condensation, and precipitation (Kelly et al., 2005). Finally, the variation of sulfur is affected by the geology of the place where the bivalve inhabits (Vinci et al., 2013). These analyses have been performed using Isotope Ratio Mass Spectrometry (IRMS), which is a relatively low cost approach and can be applied to various types of matrices; however, it is slower than some other approaches presented in the present study (Fig. 4) (Table 1).

Zhang et al. (2019a) evaluated the efficiency of using stable isotope analyses for the identification of geographic origin and the mislabeling of scallop species. Values of $\delta^{13}C$ and $\delta^{15}N$ in the adductor muscle were determined by IRMS from three different species of scallops: *Argopecten irradian*, *Azumapecten farreri,* and *M. yessoensis*. The scallops were collected at 7 locations in the Bohai Sea and the Yellow Sea (China). Stable isotope analysis was able to correctly allocate, on average about 90% of the scallops to their place of origin. Concerning species allocation, the Linear Discriminant Analysis (LDA) performed was able to correctly classify 98% of the samples. Additionally, the authors also verified the existence of significant differences in the values of $\delta^{13}C$ and $\delta^{15}N$ in samples collected in spring and autumn, demonstrating that there is seasonal variation in isotopic fingerprints, most probably due to the different composition of diets that bivalves have available over different seasons along the year. The same authors evaluated the combined use of stable isotopes and FA analyses to identify the geographic origin of the same scallop species detailed above (Zhang, et al., 2019b). Scallops were analysed by IRMS for $\delta^{13}C$ values of FA and by GC-MS for FA, and an independent LDA for each scallop species was performed for FA profiles, FA $\delta^{13}C$ fingerprints, and a combination of both. The LDA for FA profile showed a percentage of correct allocation to their place of origin of 90%, while FA $\delta^{13}C$ fingerprints achieved nearly 80%. When the two tools were combined, the percentage of correct allocation to the place of origin of the three scallop species being surveyed peaked at 100%, evidencing that the combination of the FA profile and FA $\delta^{13}C$ fingerprinting can be a precise and promising pathway to trace the geographic origin of scallops.

Another study also combined two different tools to determine the geographic origin of oysters, using stable isotope analyses and elemental analyses (Matos et al., 2021). Lead (Pb) and cadmium (Cd) concentrations were determined using ICP-MS, and $\delta^{13}C$ and $\delta^{15}N$ values using IRMS, from *C. virginica* soft tissues collected in five locations in the coasts of Florida and Texas (USA). In this study, LDA was also performed separately for each tool and when combining both. The LDA of the elemental analysis only reached a correct allocation of nearly 30%, while stable isotope analyses achieved nearly 55%. However, when the two fingerprints were combined, the percentage of correct allocation rise to nearly 70%, evidencing once more that the use of two complementary tools holds the potential to more accurately differentiate the place of origin of bivalves.

4.2.6. Unstable isotope analyses

Unstable isotopes, unlike stable isotopes, decay over time (Jafari et al., 2020). These can be radioactive or radiogenic isotopes, which are the product of radioactive decay (Bartelink & Chesson, 2019). Most unstable isotopes present in seawater can bioaccumulate in the soft and hard tissues of bivalves through adsorption, absorption, and ingestion, as some of them are chemical analogs of metabolically essential elements (Alam et al., 2000). In contrast to stable isotopes, radiogenic isotopes are not fractionated during biogeochemical processes and can assist as unique markers for traceability studies (Won et al., 2021).

The strontium radiogenic isotope ratio ($^{87}Sr/^{86}Sr$) has been used as a robust method for the traceability of the geographic origin of terrestrial food products (Bong et al., 2012; Voerkelius Susanne et al., 2010), due to its heterogeneous distribution that reflects regional geology and lithology, and little or no fractionation (Tanaka et al., 2022). Despite its wide use in terrestrial food products, this does not seem to be a good option to distinguish the geographical origin of seafood, as this ratio is quite homogeneous in the ocean (Tanaka et al., 2022). Alternatively, the neodymium radiogenic isotope ratio ($^{143}Nd/^{144}Nd$ or εNd) seems much more promising for studies aiming to discriminate the provenance of seafood (Zhao, Liu, et al., 2019), since, unlike Sr, Nd isotope is heterogeneous in the ocean, and its distribution is controlled by oceanic circulation (Tachikawa et al., 2003). Recently, studies have been carried out on the potential use of unstable isotopes for the traceability of the geographic origin of bivalves (Fig. 3) (Brombin et al., 2022; Hurtado-Bermúdez et al., 2019; Tanaka et al., 2022; Won et al., 2021; Zhao, Liu, et al., 2019), with promising results being achieved through ICP-MS, Multiple-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS), and alpha-spectrometry with Passivated Implanted Planar Silicon (PIPS) detector. This tool has high associated costs, a relatively complex methodology, and requires longer preparation and running times than other tools (Fig. 4) (Table 1) (Aggarwal et al., 2008). However, this tool only started to be used in 2019 for traceability purposes of seafood and, as this research field expands, it is likely that costs and running times become more in line with those of other tools detailed in this study.

Concentration of natural radioactive isotopes has already been studied as a fingerprint to confirm the geographic origin of cockles

(*C. edule*), clams (*Chamelea gallina*, *D. trunculus*, *S. plana*, *Solen marginatus*, *Venus verrucosa*), and mussels (*M. galloprovincialis*) (Hurtado-Bermúdez et al., 2019). The authors compared the concentrations of potassium (^{40}K), lead (^{210}Pb), thorium (^{234}Th), and polonium (^{210} Po) isotopes in the soft tissues of these bivalves using alpha-spectrometry with PIPS detector. The species were sourced from 14 different locations along the Mediterranean Sea and the Atlantic Ocean, having been possible to group their places of origin by the two large bodies of water, making it possible to claim that this tool can be used, in a rather preliminary way, to discriminate the geographic origin of bivalves at a large spatial scale.

Alternatively, Won et al. (2021) combined two tools, stable and unstable isotope analyses, to verify which isotopes were more suitable for the discrimination of the geographic origin of the clam *R. decussatus*. Clams were collected from the coasts of the Democratic People's Republic of Korea, China, and South Korea. The adductor muscle was then analysed and stable isotopes δ^{13}C, δ^{15}N, δ^{18}O, δ^{2}H, and δ^{34}S determined using IRMS, with radiogenic isotopes ^{87}Sr/^{86}Sr and ƐNd being determined using ICP-MS. The isotopes δ^{13}C and δ^{15}N were defined as standard, adding the remaining isotopes, and applying an LDA analysis that revealed that by only using stable isotopes the percentage of correct allocation to the place of origin of clams was slightly above 60%, but when these were combined with ƐNd, the percentage of correct allocation peaked at nearly 87%.

It is also worth referring the study by Zhang et al. (2019) that aimed to evaluate if by only using ƐNd as a marker in the shell of mussels *M. galloprovincialis* and *M. edulis* it was possible to correctly allocate them to their 24 places of origin along Japanese and Chinese coasts. The authors used MC-ICP-MS analysis and ƐNd values proved to be different at a national spatial scale, with mussels originating from China being successfully discriminated from those sourced from Japan. The values recorded for ƐNd also showed promise to successfully discriminate locations at a regional level, as these differed between the Yellow Sea and the Bohai Sea in China and between the Pacific Ocean and the Sea of Japan in Japan.

4.3. Trends in the study of traceability and detection of species mislabeling for marine bivalves

The last two decades have seen a considerable increase in research-oriented towards quality and methods of food safety due to consecutive food scandals, undermining consumer confidence (Power & Cozzolino, 2020). Seafood is a potential target for fraudulent practices, as processing often results in the removal of external features (e.g., bivalve shells), that make species identification increasingly difficult (Power & Cozzolino, 2020). Bivalves in particular, are not only highly prone to fraud due to species mislabeling, they are also highly vulnerable to the mislabeling of their place of origin, a feature that is paramount to safeguard consumers safety.

Scientific research on these topics has been increasing over the last two decades, with 2021 and 2022 being the years with the most published works on the traceability of geographic origin of marine bivalves and their mislabeling (Fig. 3). The technique most often chosen to pursue these goals has been DNA analysis (55%), followed by elemental analysis (21%), FA analysis (8%), stable isotope analysis (8%), metabolomic analysis (5%), and analysis of unstable isotope (3%) (Fig. 3). DNA analysis began in 2000 and has increased substantially, peaking in 2017. Moreover, since 2008, elemental analysis has also been a commonly used tool and its use has been increasing substantially until present. Concerning FA analysis, its use has peaked in recent years, with the first scientific publication reporting its use dating from less than a decade ago (2013). Conversely, metabolomic analysis has only been applied occasionally in 7 studies (2002, 2008, 2014, 2013, and 2020), while the analysis of stable and unstable isotope analysis is just now emerging as tool for this type of studies.

Scientific publications on the topics addressed in the present study have mostly originated from 23 countries worldwide (Fig. 5). Austria, Belgium, Mexico, and Poland have targeted species-mislabeling, while Brazil, Croatia, Denmark, Hungary, and Ireland have focused the traceability of geographic origin. The remaining 14 countries investigated both topics, with researchers affiliated with Spain, China, Portugal, and Italy authoring the highest number of publications. The specific interest in the investigation of these topics seems to be related with the volume of bivalves imported and/or exported by each country. Subsequently, China, Japan, the USA, France, Spain, Italy, and Portugal rank among the top 12 countries that most import bivalves (Fig. 5). Only Chile does not have a great expression in the amount of bivalves imported, however, it is the second biggest exporter of these highly priced organisms (Fig. 5). In fact, the 8 publications by Chilean researchers focus on mussels, the bivalve most exported by Chile.

Unsurprisingly, species targeted in most scientific publications are linked to the commercial relevance they play in the country of origin of those same publications (Mamede et al., 2022; Parrondo et al., 2021; Vera et al., 2019). The FAO ISSCAAP groups most addressed in the publications surveyed were clams, cockles, and arkshells (38%), mussels (29%), scallops and pectens (17%), and oysters (15%) (Fig. 6). The average commercial value of exports in 2019 reached 1.36 USD/kg for clams, cockles, and arkshells, followed by 1.19 USD/kg for scallops and pectens, 0.97 USD/kg for mussels, and 0.85 USD/kg for oysters (Fig. 6) (FAO, 2021). Although mussels have a low average commercial value, it includes mussel *M. chilensis*, one of the most produced species worldwide that can reach 7.54 USD/kg. In fact, this species was the 5th (15 articles) species most employed in scientific publications, and due to their morphological similarities *M. galloprovincialis* was the 1st (15 articles). In 2019, the clam *R. philippinarum* (4 028 163 tonnes) and the oyster *M. gigas* (653 296 tonnes) (FAO, 2021; 2022f), were the two most highly produced bivalves; coincidentally, they were also the 2nd (27 articles) and 4th (18 articles) species mostly addressed in scientific publications focusing the traceability of geographic origin and detection of species-mislabeling.

5. Conclusions

In the past two decades, the interest in the traceability of geographic origin and detection of species-mislabeling on marine bivalves has increased, with the number of scientific publications on these topics evidencing this trend. Consumers are increasingly more aware on the issues associated food fraud scandals, illegal harvesting and other unsustainable practices that degrade the marine environment and may threaten food safety standards. One way to enhance consumers trust and promote public health is to make possible to verify the claims provided in the labeling of bivalves currently being traded at a global scale, with emphasis on their identity and geographic origin. To this purpose, 6 promising tools can be currently employed, namely DNA and fatty acid analyses to expose species mislabeling, along with elemental analyses to confirm their geographic origin; additionally, the emerging use of stable and unstable isotope analyses, along as those screening the metabolome of these organisms can also be used to verify claims on marine bivalve species identification and provenance. Overall, all these tools have already revealed high levels of accuracy, but further studies are still necessary to identify how annual and/or seasonal variations can be a constraint towards the optimization of routine protocols used for the traceability of marine bivalves, so time and costs associated with the processing and analysis of samples can be reduced. Moreover, we suggest the implementation of an open database that allows researchers to immediately compare their data with that from previous studies, as this will make possible to establishing a more reliable and natural fingerprint for each marine bivalve species originating from a specific harvesting/production location.

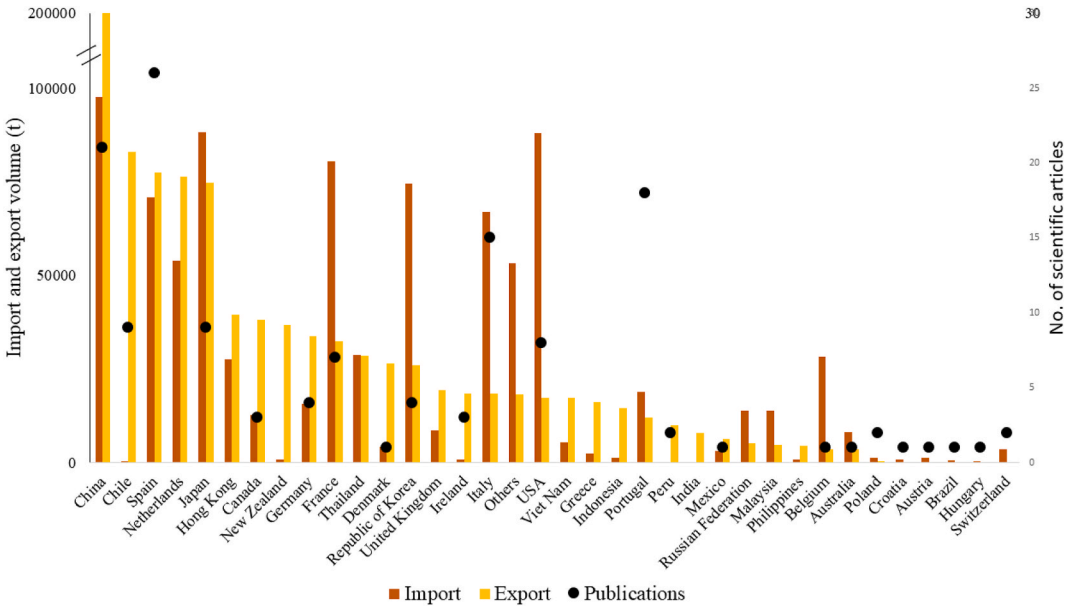

Fig. 5. Volume of exported and imported bivalves (in tons) in 2020 by country and number of scientific articles in peer reviewed literature in each country (Source: FAO). Others include the following countries: Turkey, Pakistan, South Africa, Myanmar, Faroe Islands, Taiwan Province of China, Singapore, Namibia, Nicaragua, Morocco, Belize, Ecuador, Belarus, Bulgaria, Norway, Sweden, United Arab Emirates, Mauritania, Bahamas, Slovenia, Latvia, Mozambique, Luxembourg, Jamaica, Lithuania, Oman, Tunisia, Senegal, Iceland, Iraq, Turks and Caicos Is., Greenland, Egypt, Romania, Czechia, Sri Lanka, Estonia, Côte d'Ivoire, Saudi Arabia, Samoa, Slovakia, Haiti, Honduras, Nigeria, Guyana, North Macedonia, Guatemala, El Salvador, Ukraine, Brunei Darussalam, and Panama.

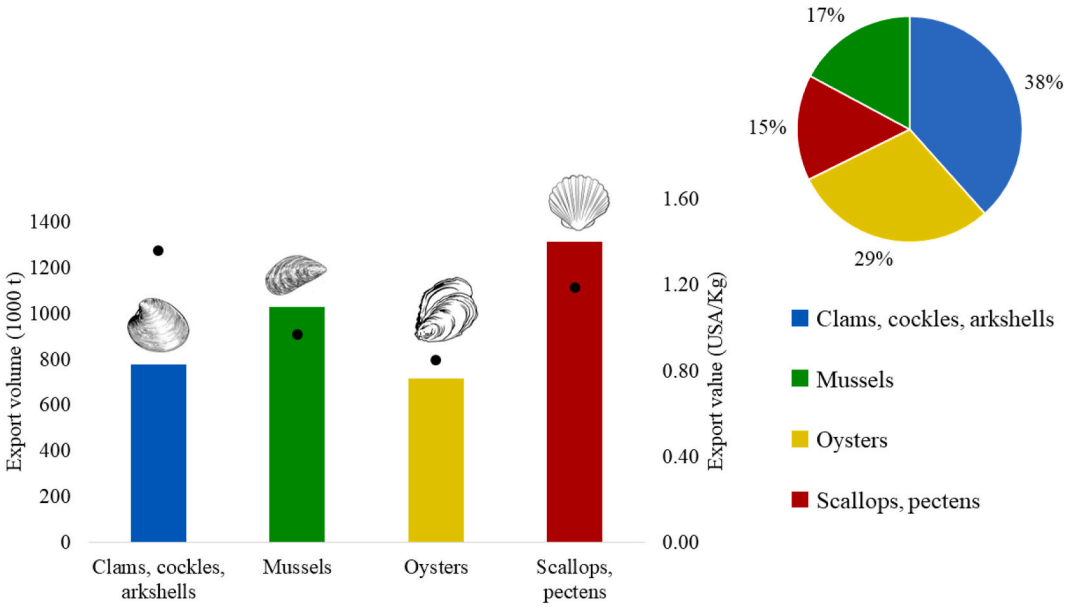

Fig. 6. Volume and average value of bivalve exports per FAO ISSCAAP groups (bar and dot graph, respectively) (Source: FAO); proportion of species in publications in international scientific journals addressing the traceability of geographic origin and detection of species-mislabeling in marine bivalves per FAO ISSCAAP groups (pie chart).

CRediT author statement

Andreia Santos: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing. **Fernando Ricardo:** Writing - Review & Editing. **M. Rosário M. Domingues:** Writing - Review & Editing. **Carla Patinha:** Conceptualization, Methodology, Writing - Review & Editing, Supervision. **Ricardo Calado:** Conceptualization, Methodology, Writing - Review & Editing, Supervision.

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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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2023.109840.

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