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Review

# Determining the provenance and authenticity of seafood: A review of current methodologies

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ABSTRACT

*Background:* Globally, food provenance has become a concern for government authorities, the seafood industry and consumers due to increasing food safety and authenticity requirements. Wild-catch fisheries and aquaculture are both important industries; aquaculture is seen as an opportunity to strengthen food security for the growing global population. However, unregulated aquaculture can expose consumers to health risks from pathogens, antibiotics and banned chemicals. Consumers and retailers, and the reputation of the global seafood industry, is affected by food fraud through species substitution and the exchange of aquaculture produce with wild-caught product and vice versa. To ensure consumer confidence and to allow authorities to effectively enforce regulations and contain risks, methods to determine the species, production methods and geographic origin of seafood need to be readily available.

*Scope and approach:* This review summarises the currently available and emerging methodologies to determine the provenance and authenticity of seafood. The main focus of this review is to give an overview of the methods that could potentially be used by authorities to enforce regulations and to contain risks, and for the seafood industry to self-regulate and protect itself from food fraud.

*Key findings and conclusions:* The most common methods used are DNA profiling, fatty acid profiling, different methods of inductively coupled plasma spectrometry and stable isotope analysis. Additionally, methods such as blockchain, radio frequency identification and x-ray fluorescence through Itrax are currently being tested for their effectiveness in determining seafood provenance. However, these methods have drawbacks and it is likely that a combination of methods would be best suited to determine the provenance of seafood considering its complex supply chain.

## 1. Introduction

Food provenance is emerging as a major concern globally for consumers, the seafood industries and regulatory bodies due to food safety issues, fraudulent relabelling of products (Ulrich et al., 2015), and a desire, by consumers, to know the origin and production methods (Kelly, Heaton, & Hoogewerff, 2005). Additionally, consumers are increasingly aware of the impact of food production on the environment and have been faced with disease outbreaks (e.g. Avian flu and white spot disease in prawns) along with malpractice from producers (Galimberti et al., 2013). These issues have compounded as countries increase food imports to focus on more profitable sectors, whilst providing consumers access to exotic and otherwise seasonal goods all year round (Marianela, Dieter, Michael, Wolfgang, & Wolfgang, 2013).

Aquaculture production has been steadily increasing since 1950 and it currently produces more seafood than from capture fisheries (FAO, 2018). If managed correctly, aquaculture can provide an important source of protein for the increasing global population (Naylor et al., 2000). Seafood is a key component of a nutritional diet because of its essential macro- and micro-nutrients, including omega-3 fatty acids and vitamins (Sioen, Matthys, De Backer, Van Camp, & Henauw, 2007; World Health Organization, 2003). The seafood market generates an estimated $151 billion USD per annum, demonstrating the high global demand for seafood (Organisation for Economic Cooperation and Development, 2017). As seafood consumption increases and wild fisheries deplete, the market will be driven towards aquaculture-based seafood products (Botsford, Castilla, & Peterson, 1997; Kearney, 2010). Consequently, the price of wild-caught seafood might increase as the

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supply depletes. This increase in price has led exploitative importers and exporters to intentionally mislabel food to profiteer by charging more for a cheaper product thus harming the reputation of legitimate businesses. Over recent years, several countries have reported this practice of substituting expensive seafood products with cheaper commodities leading to what can be described as seafood fraud (Buck, 2007; Ottavian et al., 2012). Countries, including Taiwan and Germany, have detected and protected against seafood fraud using methods such as identification of species through DNA profiling (Hsieh et al., 2010; Huang et al., 2014; Rehbein, 2008), although this approach does not determine production method. Methods of determining food provenance are required to combat food fraud, which is estimated to cost the global food industry between $30 to $40 billion USD per year (PricewaterhouseCoopers, 2016).

Apart from affecting the profitability of the seafood industry seafood fraud also raises concerns regarding the safety, hygiene and authenticity of fraudulently imported seafood (Furness & Osman, 2006; Ulrich et al., 2015). Human health has been placed at risk by the presence of pathogens and other banned substances such as antibiotics found in imported seafood (Feldhusen, 2000). More recently there have been increased concerns regarding microplastics which are being ingested by marine species consumed by humans (Choy & Drazen, 2013; de Sá, Oliveira, Ribeiro, Rocha, & Futter, 2018; Rochman et al., 2016; Rochman et al., 2015; Van Cauwenberghe & Janssen, 2014). However, it is not yet clear what effects microplastics have on human health (Rochman et al., 2016). Seafood fraud can also irreparably damage the reputation of products and their producers. For instance, if a human health issue is caused by a fraudulent product presented as a genuine one (e.g. Atlantic salmon (*Salmo salar*) being substituted with rainbow trout (*Oncorhynchus mykiss*)), it can lead to a recall of the genuine product. This can have a drastic effect on legitimate businesses that rely on exporting or importing seafood. These issues, along with the aforementioned risks to human health, highlight the need for an accurate system of tracing seafood back to its origin. Contaminated seafood can be effectively recalled if authorities can track the origin of the offending product. Furthermore, consumers have demonstrated their preference for high quality and environmentally friendly seafood (Kelly et al., 2005). Recently, there has been a public outcry over the links between seafood production and slavery along with labour rights abuse (Kittinger et al., 2017). These issues, in addition to the fraudulent mislabelling of seafood, highlight the importance of seafood provenance. The complex supply chain needed for the product to reach the consumer compounds the myriad of issues faced by the seafood industry (Leal, Pimentel, Ricardo, Rosa, & Calado, 2015). Therefore, accurate methods to determine seafood origin and production methods are necessary to ensure consumer confidence in imported seafood.

There are several methods that are currently available for determining the provenance of seafood, along with a few emerging methodologies. Previously there have been several reviews that focused on highlighting the applicability and potential shortcomings of several of these methods (Leal et al., 2015; Primrose, Woolfe, & Rollinson, 2010; Verrez-Bagnis, 2017). However, there are only a limited number of studies exploring emerging technologies in food provenance and fewer still exploring their role in determining the provenance of seafood (Badia-Melis, Mishra, & Ruiz-García, 2015; Schröder, 2008). Therefore, a systematic review which covers the advantages and disadvantages of the current methods is necessary in order to compare them with emerging technologies which are being tested for their use in determining seafood provenance. In this review, we highlight some important limitations and constraints, both technical and commercial, and identify instances along the food supply chain where seafood fraud can occur. Our aims are, therefore:

1) To provide an updated overview of the methods available to determine seafood provenance;
2) Highlight new and emerging technologies that are currently being studied for their use in determining seafood provenance; and
3) To provide practical recommendations regarding the appropriate application of methods.

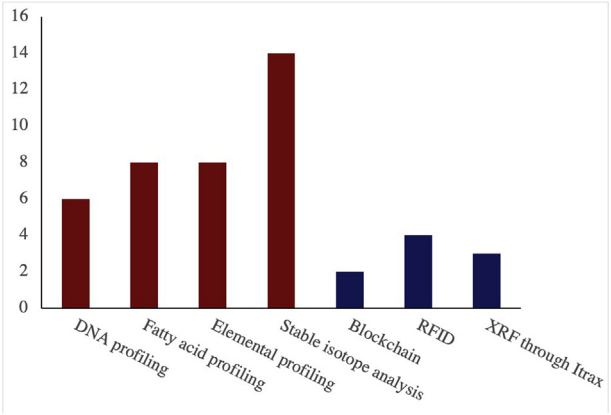


**Fig. 1.** Bar graph showing the number of keystone studies examined in this review for each method from 1990 to 2019. All of the papers in this graph examined the use of methods to determine the provenance of seafood.

## 2. Methodology

A literature search was conducted using Boolean search terms on 'Elsevier', 'Science Direct', 'ProQuest' and 'Scopus' to find papers from the last 20 years of research that were relevant to the literature review. The Boolean search terms included the name of the methodology (including abbreviations) along with the term 'traceability OR provenance OR fingerprint OR authentication'. This was done to ensure that the search captured a wide range of papers that may have used different keywords. From this search, "keystone" papers that indicate the efficacy of a method were examined, and their contents were analysed (Fig. 1). The terms 'traceability OR provenance OR fingerprint OR authentication' were chosen as they were commonly used interchangeably in the literature. Generally, the keywords 'traceability', 'fraud', 'safety', and 'authentication' appeared together with some papers using 'fingerprint' or 'provenance' in place of 'authentication' and 'traceability'.

## 3. Results and discussion

### 3.1. DNA profiling

DNA profiling has been used extensively by several producers and authorities to determine the provenance of food (Scarano & Rao, 2014), due to the many advantages provided by DNA profiling. Using DNA profiling to identify animal species in food is ideal because the behaviour of DNA is not species-dependent and is predictable, food samples which have been heated up to 120 °C in the cooking process can still be analysed, and the diversity of DNA allows for the differentiation of closely-related species and even subspecies (Lenstra, 2003). Determining the species of plants or animals used in food products is an important part of detecting food adulteration as well as allowing for the detection of food substitution (Hsieh et al., 2010; Huang et al., 2014; Rehbein, 2008).

Generally, DNA profiling is based on two types of markers; hybridisation-based markers and Polymerase Chain Reaction (PCR)-based markers. Out of these two methods, PCR is considered faster and more accurate when compared to other methods (Labra et al., 2004; Teletchea, Maudet, & Hänni, 2005). There is a variety of other methods also used for DNA profiling in food traceability; however, the lack of standardisation and universality of methods is an issue (Galimberti et al., 2013). This is especially important as new species are introduced into the market as demonstrated by Rehbein (2008). When these

species are introduced to new markets it is likely that they will also be subject to seafood fraud. For example, high-value species such as arrowtooth flounder (*Atheresthes stomias*) and yellowfin sole (*Limanda aspera*) were being substituted with cheaper species known as pangasius (including *Pangasius hypophthalmus* and *Pangasius bocourti*) which were recently introduced to the European market. Rehbein (2008) focused on profiling these cheaper species to detect seafood fraud and noted that without a centralised public database it is difficult to use DNA profiling as a method for detecting food substitution. However, this is currently being addressed with databases such as the Barcode of Life Data System (BOLD) and the Fish Barcode of Life (FISH-BOL) (Barbuto et al., 2010).

DNA profiling has been used to investigate several instances of food substitution in the seafood market (Barbuto et al., 2010; Carrera et al., 2000; Cross & Challanain, 1991; Russell et al., 2000). Barbuto et al. (2010) collected 45 samples of "palombo" (*Mustelus mustelus* or *Mustelus asterias*) from the Italian market and characterised their DNA profile using PCR. When the profiles were compared to samples in BOLD, it was found that 35 out of the 45 samples were substituted with a different species. Only three of the collected samples could be related directly to "palombo" (they were all *M. mustelus*). While this study demonstrates that DNA profiling can determine the species of a product to help prevent seafood fraud, it also highlights the lack of standardised databases containing the DNA profile of different species of seafood. Thus, the DNA profile of the majority of seafood species need to be analysed and curated. Nevertheless, the method is able to show that a seafood product has been mislabelled or substituted.

Similarly, Carrera et al. (2000) used PCR to identify and compare the DNA profile of Atlantic salmon (*S. salar*) and rainbow trout (*O. mykiss*). The main purpose of this study was to determine if DNA profiling could differentiate between these two species because *S. salar* is often substituted with *O. mykiss*, as it is a cheaper product. The study showed that DNA profiling could cost-effectively discriminate between these two species. The study also determined that the DNA profile of smoked samples was similar to the raw samples for both species, demonstrating the robustness of DNA. Additionally, Russell et al. (2000) used DNA profiling to differentiate between ten different species of salmon from a number of different locations. This study focused on using reactive enzymes to trim the DNA down to specific locations that differentiated between the species. Russell et al. (2000) were able to differentiate the species using a DNA fragment that can be amplified even in processed samples. This shows that the current process of DNA profiling can be further improved while reducing the cost of analysis and improving throughput. Hsieh et al. (2010) were able to replicate this process for four species of puffer fish. The method was less expensive than a direct sequencing analysis and was able to produce results within 9 h. The result suggests that this methodology is ideal for use in seafood identification, especially when a rapid analysis is required for regulatory bodies to make decisions. Huang et al. (2014) further expanded on this methodology by determining the gene markers for 12 different species of puffer fish and adding them to the GenBank database. The authors remarked that the short gene marker regions were remarkably stable against environmental stress and can be used with PCR. This is especially important once the species are processed and used as part of other products. These studies show the benefits of utilising PCR and characterising certain sections of DNA, the cost and benefit analysis of each method is important as food fraud already costs the food industry a large amount of money. Therefore, methods which can be used to detect and prevent food fraud need to be accurate while being affordable.

In addition to differentiating between species, it is also important to differentiate between the same species from different production methods. This is especially important for species like *Lates calcarifer* as they are cultured and wild-caught in multiple countries (e.g. Australia, Malaysia, Indonesia, Taiwan, etc) and sold under different names in certain markets (e.g. Asian seabass vs Barramundi) (FAO, 2006). Cross and Challanain (1991) successfully discriminated between farmed and wild-caught *S. salar* from Ireland. The farmed samples were genetically different as most of the fingerlings for the farming stock were likely from Norway or Scotland, while the wild-caught samples were genetically adapted to Ireland. The exact cause of genetic variation between the same species has not been researched exhaustively and some studies argue that the variation is caused by adapting to local conditions while others argue that it is caused by genetic drift (McGinnity et al., 1997). Although DNA markers may vary according to different geographic locations, the causes of genetic variation between the same species need to be explored to determine whether or not DNA profiling can be used as a method for determining the production method of seafood.

### 3.2. Fatty acid profiling

Fatty acids are aliphatic monocarboxylic acids which are contained in, or derived from, an animal or vegetable fat, oil or wax. Natural fatty acids can be saturated or unsaturated and contain 4 to 28 carbons (Nic, Hovorka, Jirat, Kosata, & Znamenacek, 2014). Fatty acid profiling typically uses various methods of chromatography or spectroscopy to determine the fatty acid composition of samples (James et al., 2011). However, in seafood provenance research, it is typically bundled with Stable Isotope Analysis (SIA) (Busetto et al., 2008; Zhang, Liu, Li, & Zhao, 2017). While this method is slower than some of the others due to the time taken to prepare samples for analysis (Budge, Iverson, Bowen, & Ackman, 2002), it has shown promise in discriminating between production methods and geographic origin of samples (Bergström, 1989; Grahl-Nielsen, Jacobsen, Christophersen, & Magnesen, 2010; Grigorakis, Alexis, Taylor, & Hole, 2002; Nemova, Fokina, Nefedova, Ruokolainen, & Bakhmet, 2013; Olsen, Grahl-Nielsen, & Schander, 2009; Ricardo et al., 2015b).

The dietary lipids of penaeid shrimp are reflected in the fatty acid composition of samples. This was demonstrated by Lim, Ako, Brown, and Hahn (1997) who examined the differences in the fatty acid composition of *Penaeus vannamei* fed seven different diets. While the aim of the study was not directly related to seafood provenance, it provides a basis for discriminating between shellfish. The diets which contained high levels of unsaturated fatty acids caused the fatty acid profile of the *P. vannamei* to differ. This suggests that the differences in diets between farms and wild-caught shellfish should vary significantly, allowing for them to be distinguished. Budge et al. (2002) performed a large-scale study which analysed the fatty acids of 28 different species of fish and invertebrates from three geographic locations. Using fatty acid profiling they were able to determine the provenance of 16 of these species with an accuracy greater than 98%. While they were able to distinguish between geographic origin using fatty acid profiles influenced by diets, the variation within-species was not as high as that of among-species variation, suggesting that differentiating between production methods may be difficult. This paper showcases the utility of fatty acid profiling in distinguishing between different geographic locations of seafood. Grigorakis et al. (2002) analysed the fatty acids on gilthead sea bream (*Sparus aurata*) using a VARIAN 3300 gas chromatograph. While this study was not specifically aimed at determining the provenance of *S. aurata*, it still showed that there was a significant difference in the lipid and fatty acid profile of wild-caught samples compared to farmed samples. In all cases, it was found that the farmed samples had higher lipid content than their wild counterparts. Additionally, it is important to note that the fatty acid profile of the farmed samples reflected the composition of their feed. This shows that fatty acid profiling can also be suitable for distinguishing between farmed and wild-caught fish. However, Grigorakis et al. (2002) noted that there was a significant seasonal variation in wild-caught *S. aurata* samples. This could become a potential issue if the samples' fatty acid profile matches that of one from an entirely different region due to these seasonal variations. This is a well-known issue and was already highlighted by Bergström (1989). The study by Bergström (1989) showed that the seasonal changes in fatty acid composition and total lipid content of wild and farmed *S.*

*salar* vary significantly before smolting. Smolting is a process by which the salmon undergoes morphological changes before migrating to the sea (McCormick, Hansen, Quinn, & Saunders, 1998). The wild-caught *S. salar* had a significantly higher concentration of saturated fats than the farmed *S. salar*. The study also found that some polyunsaturated fatty acids were higher in wild-caught samples when compared to farmed samples. Additionally, significant changes were found in the total lipid content of both farmed and wild-caught fish between seasons. While Bergström (1989) did not aim to discriminate between the farmed and wild-caught salmon, the results are still significant for food traceability. The results from this study suggest that there are significant seasonal changes to the lipid content of fish and that this needs to be considered when attempting to trace the geographic origin and production method of seafood using fatty acid profiling. Nemova et al. (2013) were able to show that the fatty acid profile of the gill lipids in blue mussels (*Mytilus edulis*) varied greatly with salinity. This variation in the fatty acid composition can be used to distinguish between different production methods such as intertidal zone collection or aquaculture. The results from these studies suggest that fatty acid profiling can be a valuable tool to trace the production method of seafood.

Ricardo et al. (2015b) used the fatty acid profile of cockles (*Cerastoderma edule*) to determine if it could distinguish between eight different sites which were from a single coastal lagoon in Portugal. The study found that fatty acid profiling was able to discriminate between collection sites within the same coastal region. However, it was unable to discriminate between the same production areas. It is important to note that these sites are defined by the amount of *Escherichia coli* present in the flesh of the *C. edule*. Therefore, discriminating between these collection and production areas can be an important marketing tool for the *C. edule* industry as it will be able to prove that the product is of higher value due to a lower amount of contamination. Additionally, Ricardo et al. (2015b) note that the method can be even more cost effective by focusing on a particular part of the cockles. Olsen et al. (2009) used fatty acid profiling to differentiate clams (*Astarte sulcata*) between four areas, grouped according to proximity, in Norway. The study found that fatty acid profiling is advantageous when comparing closely-related sites as it gives high-resolution results, allowing it to detect small changes in populations that are geographically close. However, it is important to note that the composition of the fatty acids present in the samples is what discriminates between sites and not the presence or absence of certain fatty acids. Similarly, Grahl-Nielsen et al. (2010) used fatty acid profiling to discriminate between five locations along the coastal regions of Norway. The data collected in the study were analysed using Partial Least Square (PLS) and Principal Component Analysis (PCA) to distinguish between groups. The study found that the five sites had distinct fatty acid compositions, further supporting the conclusion drawn by Olsen et al. (2009). Hence, it is clear that fatty acid profiling can play a distinct role in distinguishing samples from geographical locations.

Fatty acid profiling has also been used in conjunction with SIA to distinguish between the geographic origins of seafood. A study conducted by Busetto et al. (2008), comparing the fatty acid composition of wild and farmed turbots, found that the monounsaturated and polyunsaturated fatty acids of the farmed fish were higher than in wild-caught fish. This would enable a measurable difference in the fatty acid composition of the two production methods. Both the fatty acid and SIA results were used in a PCA to discriminate between geographic locations and the production methods of turbots. Similarly, Zhang et al. (2017) used fatty acid profiling and SIA of carbon and nitrogen to differentiate between sea cucumbers (*Apostichopus japonicus*) collected from seven sites in northern China. While the stable carbon and nitrogen values were capable of distinguishing between five out of the seven sites, it had an overlap between two, likely due to the fish having similar food sources. However, by using both the stable isotope values and fatty acid profiles, the PCA was able to distinguish between the seven sites clearly.

### 3.3. Elemental profiling

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is a high-precision method used to detect the concentrations of trace elements in geological samples (Jenner, Longerich, Jackson, & Fryer, 1990; Reid, Horn, Longerich, Forsythe, & Jenner, 1999). ICP-MS has been utilised in the field of food traceability and can distinguish between the geographic origins of seafood (Dunphy, Millet, & Jeffs, 2011; Ricardo et al., 2015a; Sorte, Etter, Spackman, Boyle, & Hannigan, 2013). This method requires samples to be digested before being analysed and certain elements, such as mercury, may require further preparation (Hight & Cheng, 2006; Ricardo et al., 2015a). Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) also known as Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) is similar to ICP-MS and has also been utilised in seafood provenance studies (K. A. Anderson, Hobbie, & Smith, 2010; Li, Boyd, Odom, & Dong, 2013; Smith & Watts, 2009). These methods can be performed on both soft tissue and hard calcified tissue; the methodology and interpretation of results are different according to the type of tissue sample being examined (K. A. Anderson et al., 2010; Dunphy et al., 2011).

Sorte et al. (2013) used laser-ablation inductively-coupled mass spectrometry (LA-ICP-MS) to determine the geographical origin of blue mussel (*Mytilus edulis*). The study was able to distinguish between five different sites, roughly 50 km apart, with greater than 50% accuracy in all cases. The accuracy was higher for juvenile samples and when close sites were grouped together it increased the accuracy to 97%. Similarly, Dunphy et al. (2011) used LA-ICP-MS to assign juvenile mussels to their collection sites around northern New Zealand with over 60% accuracy in all cases. Seven elemental ratios were proven to be the most reliable when used with discriminant analysis. This study is important as it demonstrates that not all elements need to be determined when distinguishing the provenance of seafood samples. Ricardo et al. (2015a) used ICP-MS to determine the concentration of aluminium, barium, calcium, cadmium, copper, magnesium, manganese, lead, strontium and zinc in cockle *(Cerastoderma edule)* shells. These 10 elements were analysed using three different statistical analyses and it was found that these elements were capable of distinguishing samples as little as 1 km apart. Cubadda, Raggi, and Coni (2006) used ICP-MS to determine the elemental concentration of 16 different elements present in Mediterranean mussel (*Mytilus galloprovincialis*) and a few other species of fin-fish. Using PCA they were able to clearly distinguish between three farming sites in close proximity. Similarly, Costas-Rodríguez, Lavilla, and Bendicho (2010) determined 40 elements in 158 samples and used it to distinguish between five collection sites of *M. galloprovincialis*. The study used linear discriminant analysis (LDA) and found that reducing the number of elements down to 16 reduced the accuracy down to 95.6% but had a number of incorrect predictions.

ICP-AES has also been utilised as a method for determining the elemental composition of samples to differentiate between geographic locations. K. A. Anderson et al. (2010) used a combination of ICP-AES and SIA to differentiate between farmed and wild-caught king salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*) and Atlantic salmon (*Salmo salar*). Using PCA and Canonical Discriminant Analysis (CDA) it was possible to discriminate between the production methods of each species using only 12 elements. This demonstrates the efficacy of the ICP-AES methodology in determining the provenance of seafood. Similarly, Li et al. (2013) utilised ICP-AES to discriminate between three different locations of channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*) using 11 elements. While the study was able to successfully discriminate the geographic origin of the samples, it was noted that the elemental profile could vary or even be similar across various locations with similar elemental compositions. Additionally, as the elemental composition of water will vary within the same geographic origin, it is important to have a large database of the elemental composition of a species for a given country, before elemental profiling can be used for seafood provenance (Li et al., 2013). A

similar issue was raised by Smith and Watts (2009) when they undertook a large scale study to determine the provenance of prawns (mostly *P. vannamei* and *Penaeus monodon*) from 8 countries. The samples were collected from around ten regions in each country and analysed using ICP-MS. The overall accuracy of the database was around 90% and the study calls for databases to be built for different geographic origins to provide a comparative base to allow for accurate prawn provenance determination (Smith & Watts, 2009).

### 3.4. Stable isotope analysis

SIA uses isotopes to distinguish between samples. Isotopes are elements which have the same number of protons but different numbers of neutrons. Stable isotopes are assimilated into animal tissues as they move up through the trophic chain (Fry, 1991; Mazumder, Wen, Johansen, Kobayashi, & Saintilan, 2016; Peterson & Fry, 1987; Post, 2002). Typically, the technique determines the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) but hydrogen and oxygen can also be detected. Primary producers have a distinct isotopic signature and when they are consumed by animals this signature assimilates into the tissues of the consumers through a process known as fractionation, which is caused by the changes in the heavy to the light isotopic ratio (Ehleringer, Rundel, & Nagy, 1986). The stable carbon isotopes indicate the sources of nutrients, while the nitrogen isotopes indicate the trophic level of an organism in the food web. Because the isotopic values of a consumer are related to the composition of the diet (Fry, 2006; Kling, Fry, & O'Brien, 1992), the differences in such diets, stemming from changes in farming practices or environmental conditions, would be reflected in the isotopic profile of consumer's muscle.

SIA for food provenance has shown positive results when discriminating between production methods (Carter, Tinggi, Yang, & Fry, 2015; Gamboa-Delgado et al., 2014; Kim, Kumar, & Shin, 2015; Ortea & Gallardo, 2015; Turchini, Quinn, Jones, Palmeri, & Gooley, 2009). Molkentin, Meisel, Lehmann, and Rehbein (2007) utilised SIA of the $\delta^{13}C$ and $\delta^{15}N$ ratio to differentiate between conventionally farmed, wild caught and organically farmed Atlantic salmon. However, SIA alone was not able to distinguish between the three and the studies found that a combination of linoleic acid and SIA was necessary to distinguish between the three different production methods of the Atlantic salmon. Molkentin, Lehmann, Ostermeyer, and Rehbein (2015) used stable isotopes and fatty acid analysis to distinguish between the organic and conventionally farmed Atlantic salmon. This study showed that it was possible to distinguish between the production methods of Atlantic salmon using SIA alone. Similarly, Fasolato, et al. (2010) used $\delta^{13}C$ analysis of fat-free muscle to successfully distinguish between farmed and wild-caught *L. calcarifer*, as the $\delta^{13}C$ shows the feeding habit of the fish. Fasolato et al. (2010) selected $\delta^{13}C$ based on the study by Sweeting, Barry, Polunin, and Jennings (2007) who concluded that $\delta^{13}C$ has minimal seasonal variation, especially in large predatory fish. A recent study by Gopi et al. (2019b), showed that SIA alone was able to determine the provenance of *L. calcarifer* with over 84% accuracy and only had two incorrect predictions out of six. This suggests that SIA, like many of the other methods, can distinguish the provenance of seafood. However, Gopi et al. (2019b) found a significant enrichment in the $\delta^{15}N$ values of wild-caught *L. calcarifer*. This finding conflicts with the findings of Serrano, Blanes, and Orero (2007) as they found no significant enrichment of $\delta^{15}N$ values for wild-caught gilthead sea bream (*Sparus aurata*). The difference in enrichment could be due to interspecies variability, as the studies analysed data from two different species. However, the findings of Gopi et al. (2019b) agree with Moreno-Rojas, Tulli, Messina, Tibaldi, and Guillou (2008) who analysed rainbow trout (*O. mykiss*) using SIA to discriminate between samples fed with fishmeal and plant-based protein diets. The study found that the samples fed the fishmeal were significantly enriched in $\delta^{15}N$ due to the fish-based proteins present in their diet.

On the other hand, other studies which have used SIA to determine the geographic origin of samples found that utilising SIA alone was not always accurate (Carter et al., 2015; Turchini et al., 2009). Further studies are needed to investigate the application of SIA combined with other analytical techniques to assess if the approach can determine the geographic region of samples accurately. Kim, Kumar, Hwang, et al. (2015) used SIA to differentiate between the geographic origin of shrimp and hairtail fish. The study found that the Korean hairtail fish (family Trichiuridae) and their international counterparts had significantly different isotopic signatures for both $\delta^{13}C$ and $\delta^{15}N$. This suggests that SIA can be used to discriminate between the same species from various geographical locations. Gamboa-Delgado et al. (2014) used SIA to differentiate between wild-caught and farmed Pacific white shrimp (*Litopenaeus vannamei*) where there was more variability in the $\delta^{13}C$ values of the wild-caught and farmed shrimp than in their $\delta^{15}N$ values. The variability of the $\delta^{15}N$ values was significantly lower in the farmed samples than in the wild-caught samples. In general, the samples which were wild caught were isotopically enhanced for both $\delta^{13}C$ and $\delta^{15}N$. Using both the $\delta^{13}C$ and $\delta^{15}N$ isotope values to discriminate between the samples is ideal as it had an accuracy of 99% in this study.

SIA is not without fault as noted by a study conducted to discriminate between cultured and wild caught turbot (*Psetta maxima*). The SIA results showed overlap between the carbon and nitrogen ratios of wild-caught Danish turbots and cultured Spanish turbots (Busetto et al., 2008). This argument is supported by a number of studies such as the review conducted by Primrose et al. (2010), which suggests that SIA should be combined with trace element measurements to provide a comprehensive method for seafood traceability. Ortea and Gallardo (2015) found that combining the carbon and nitrogen stable isotope ratios along with other elements such as arsenic and lead is the most reliable method of tracing the production method and geographic origin respectively. Carter et al. (2015) used SIA to analyse both the water recovered from prawn samples as well as the prawns in order to distinguish between Australian and imported prawns. The water had no correlation with their respective tissue samples and using the results obtained from the water was not sufficient to distinguish between Australian and imported samples; the isotopic analysis revealed significant differences in hydrogen and carbon isotopes. Turchini et al. (2009) used chemical analyses as well as carbon, nitrogen and oxygen stable isotopes to discriminate between different farms. This study showed the effectiveness of SIA in differentiating between farms in the same region. However, a study conducted on wild-caught and farmed Brazilian freshwater cachara suggested that the C/N ratio of these fish varies seasonally, especially in the wet season. However, the C/N ratio was indistinguishable in the dry season, suggesting that the farmed and wild-caught samples would be indistinguishable during this time period. The lack of variation between the production methods needs to be studied to determine the cause (Sant'Ana, Ducatti, & Ramires, 2010). Gopi et al. (2019b) used both SIA and elemental profiling, through X-ray fluorescence, and found that a combined model built using both datasets had an accuracy of 81% and had no incorrect predictions out of six. This result was repeated by Gopi et al. (2019a) for black tiger prawns (*P. monodon*) where the combined dataset resulted in an accuracy of > 98%. Both these studies showed that SIA can be utilised in conjunction with other analytical techniques to provide an accurate tool to authenticate the provenance of seafood. We believe that the higher accuracy shown in both Gopi et al. (2019b) and Gopi et al. (2019a) is due to the way in which they analysed the data; both studies used multiple statistical analyses designed to distinguish between groups to determine the provenance of their samples, leading to an increased overall accuracy. While these results are promising, the methodology needs to be studied further as they found variations in the results depending on the species.

## 4. New and emerging technologies and methodologies

### 4.1. Blockchain

The blockchain technology was exposed to the world through the bitcoin cryptocurrency in 2008 (Bhardwaj & Kaushik, 2018). Blockchain technology involves a combination of principles from the 1960s based on timestamping digital documents using crypto signatures (Haber & Stornetta, 1990), a decentralised storage system where records cannot be purged (R. Anderson, 1996) and encrypting files to prevent access from untrusted/unauthorised machines (Schneier & Kelsey, 1998). Hence, blockchain has the ability to operate without a centralised and trusted authority to authenticate transactions or records and prevents them from being erased. Additionally, the system is made secure from unauthorised parties through the use of mathematical problems, which needs a substantial amount of computational power to solve (Galvez, Mejuto, & Simal-Gandara, 2018).

The advantage of utilising blockchain over traditional bookkeeping methods is the ability to encrypt end-to-end traceability and allow the consumer to access this information easily via the internet (Galvez et al., 2018). When this technology is applied to the food supply chain it will allow for the storage of a wide range of data, from GPS coordinates of where fish were caught to the batch number of a fish produced through aquaculture. Any data that are deemed to be important can be added into the blockchain system by the members of the business network. Once all members of the network authenticate the entered data they cannot be altered, allowing for a permanent record of all data on that particular food product (Galvez et al., 2018). Some businesses, like Walmart in the USA, are already in the process of implementing blockchain into their produce (Yiannas, 2018). When the methodology was applied to their mango supply chain, the time taken to trace the end product back to the farm reduced from almost seven days using traditional methods down to 2.2 s using blockchain (Yiannas, 2018). The blockchain cannot only keep an immutable copy of records but can also make accessing those records much more efficient.

Implementing blockchain into the seafood supply chain can be a relatively simple and cost-effective process because of cloud-based models (Korpela, Hallikas, & Dahlberg, 2017). As an example, a wild-catch supplier of skipjack tuna (*Katsuwonus pelamis*) would enter the catchment area of a batch of fish which is then transported to the processor. The primary processor would then fillet the fish, ensuring that this process is entered into the blockchain before shipping the produce to a cannery. The blockchain of final product (i.e. canned tuna) would contain all this information, which can then be easily accessed by regulatory bodies and industry partners. An added benefit of this technique of bookkeeping, is that this information can be passed along to the consumer, allowing them to make informed decisions. The main advantage of using blockchain for seafood provenance is that it is tamper proof. To falsify a record entered into a blockchain system, you would need control of over 51% of the nodes in the supply chain (Tian, 2018). When a new transaction or record is added into the system it is verified by the nodes in the whole system, before it is added to the blockchain (Tian, 2018). However, for the blockchain system to work in a real-world context, it needs to be taken up by every single organisation along the supply chain. If it is not implemented in this manner it would make it difficult for the chain of custody of a seafood product to remain tamperproof.

As mentioned previously, the seafood supply chain is incredibly complex (Leal et al., 2015). The initial product can go through multiple processors and supply chain actors before it reaches the consumer, and along the way some information can become lost. For instance, a secondary producer might obtain the same species of fish from multiple sources and fillet them before sending it to the distributor. If a box of fillets is almost full then it is likely that it will be filled with fillets from other sources. This information might not be stored in the blockchain as fillets are indistinguishable from each other even if they are from multiple sources. Therefore, to ensure that all partners are complying with the correct procedures necessary for blockchain, a method is required for testing the actual provenance of seafood. This can act as a deterrent for food fraud and provide additional information to consumers so that they can be assured that they are purchasing a properly labelled product.

### 4.2. Radio frequency identification

Radio frequency identification (RFID) is also an emerging provenance technology. RFID stores information, such as serial numbers and place of origin, on a microchip attached to an antenna. The information emitted through the radio waves is scanned and converted to digital information which can be displayed on the scanner (Doukidis, Pramatari, & Kelepouris, 2007). The advantage of utilising RFID is that it can automatically capture data needed for traceability with minimal changes to the business process, leading to reduced expenditure needed to implement the system (Doukidis et al., 2007). RFID does provide certain advantages over traditional barcodes such as being automatic, having a long lifespan, being robust and being able to store up to 32 kilobytes of data in each tag (Costa et al., 2013). RFID also offers some advantages such as being hidden out of sight as the tags do not need to be in sight of the reader to work, allowing for them to be stored inside the product or containers (Costa et al., 2013). Additionally, in the majority of environments RFID can be read successfully in the first scan in 99.5%–100% of cases (Texas Instruments, 2006). In Europe, this system has been implemented by several seafood producers as part of their compliance with European regulations on traceability (Schröder, 2008). Furthermore, the technology has also been used to monitor the temperature fluctuations inside a refrigerated vehicle transporting frozen tilapia fillets (Tingman, Jian, & Xiaoshuan, 2010). This would allow retailers to predict the shelf-life of the product based on temperature fluctuations (Tingman et al., 2010). As food traceability requires all partners in a supply chain to work cooperatively, it might be expensive for small and medium-sized enterprises to implement a system on par with the bigger enterprises. RFID can play a significant role in cases like these as a potential traceability system which uses RFID might only require a scanner and a computer (Doukidis et al., 2007). Therefore, RFID is a potentially cost-effective solution which can be compete with methods such as blockchain. However, this does not prevent seafood fraud as RFID chips with fraudulent information can be created easily. Therefore, methods which can authenticate the production methods and geographic origin of seafood are necessary to ensure that products are correctly labelled using RFID.

### 4.3. X-ray fluorescence through Itrax

X-ray fluorescence (XRF) through Itrax is a method that provides the elemental composition of samples and is typically utilised for sediment core scanning or dendrochronology (Gunnarson, Linderholm, & Moberg, 2011; Keegan et al., 2008; Zuo, 2013). XRF through Itrax has the potential to be a cost-effective method of determining the elemental profile of seafood samples. While it may not give quantified values of elements, it can detect the presence of up to 31 different elements and should be readily available in geology labs.

Recently, Gadd, et al. (2018) developed a method that determines the elemental composition of organic soft-tissue samples. This method was used to developed a seafood provenance model for Asian seabass (*L. calcarifer*) (Gopi et al., 2019b). This study managed to correctly classify both the geographical origin and production method of *L. calcarifer* samples with no incorrect predictions using both SIA and elemental data gathered using Itrax. A similar approach was taken for tiger prawns (*P. monodon*) to distinguish the provenance of the samples with high certainty (Gopi et al., 2019a). However, while these results are promising, it is important to note that the datasets used to train the models in these two papers were limited. The differences between the

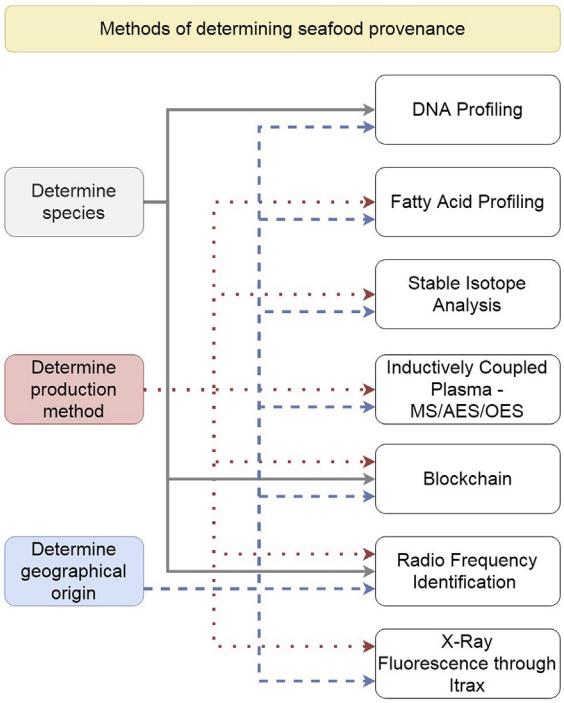


**Fig. 2.** Decision tree to choose a method suitable for determining seafood provenance and authenticating species for different scenarios.

different geographic origins and production methods need to be explored further. Once the causes of differences are determined the knowledge can be used to adjust and train the model further to reduce incorrect predictions. Currently it is unclear whether or not the interpretation of the results will change according to the type of tissues being scanned because the studies have only used muscle tissue. Therefore, this method should be tested on more species and different types of tissues in order to determine its utility as a tool for seafood provenance.

### *4.4. The current state of seafood provenance*

Currently, regulatory bodies and authorities have several methods available to them to determine the provenance of seafood. New methodologies are constantly being developed and can often supplement the current suite of tools. Therefore, to determine the ideal method for different scenarios the decision tree we developed can be consulted (Fig. 2). Furthermore, to understand the advantages and shortcomings of each method a table has been provided summarising the findings of this review (Table 1).

DNA profiling has clear advantages when it comes to detecting food substitution as the species of even heavily processed samples can be determined. While the issue of universality is being tackled by databases such as BOLD and FISH-BOL (Barbuto et al., 2010), the issue of standardisation still exists. Standardisation is a much more difficult issue to tackle as it requires all labs to follow a standard procedure. Even with these drawbacks, DNA profiling remains one of the most recommended methods of analyses when it comes to detecting food fraud, at least for determining the species. However, the inability of DNA profiling to distinguish between farmed and wild-caught samples of the same species, that are from the same location, makes it difficult to use when farmed samples are passed off as wild-caught.

Fatty acid profiling has been used to distinguish the provenance of a wide variety of seafood. It is not only useful for distinguishing between production methods but also to discriminate between geographical origins (Busetto et al., 2008; Nemova et al., 2013). The high-resolution output provided by fatty acid profiling can distinguish between seafood from similar locations more effectively than by SIA. However, the seasonal variability in the fatty acid composition of different species needs to be explored. If this is not done, then there can be potential overlaps between geographical locations and production methods. Additionally, while there are variations to the fatty acid compositions according to both production methods and geographic locations, it could potentially be manipulated through controlling the diets. Furthermore, to determine the actual origin of unknown samples, a database containing the fatty acid profiles of different species is needed. Once these issues have been addressed, fatty acid profiling can play an important role in seafood provenance.

ICP-MS or ICP-AES has been shown to be an important tool for seafood provenance (K. A. Anderson et al., 2010; Sorte et al., 2013). While ICP-MS can determine the concentration of most elements, some studies attempted to reduce the number of elements needed to clearly distinguish between locations. This is a key issue that needs to be addressed, as ICP-MS requires samples to be prepared before analysis and cannot measure all elements in one sample run. Therefore, determining a large number of elements in each sample will require a significant amount of time. For ICP-MS to become a standard methodology for discriminating between the geographical locations and production methods of seafood, the elements necessary to discriminate the provenance of each species needs to be determined. Additionally, a database of the elemental profile of a species from a geographic region needs to be recorded to allow for comparison of samples to determine food fraud. Once these issues are addressed, elemental profiling can play a role in fingerprinting the geographic locations of seafood.

SIA is differentiated by being able to distinguish not only the geographic origin of seafood but also the production method with a relatively high accuracy (Gopi et al., 2019b; Molkentin et al., 2007). However, most of the studies had to use another method to distinguish between samples that had overlaps in their stable carbon or nitrogen isotopic ratios (Busetto et al., 2008; Ortea & Gallardo, 2015). These overlaps may occur due to farmed species being fed a similar diet, wild-caught species migrating across borders and anthropogenic pollution. Additionally, seasonal variability in the stable isotopes may cause these overlaps to occur. As the isotopic composition of a product is influenced by factors such as feed and environmental conditions, it is possible that the isotopic composition can be manipulated by controlling these factors. While this type of fraud is unlikely to occur due to the costs involved, it would make detecting these instances of fraud difficult using SIA alone. Therefore, from our experience and from the literature (Gopi et al., 2019b, 2019a; Primrose et al., 2010), it is suggested that SIA is combined with other methods when used for seafood provenance as it helps to distinguish between samples with the same isotopic signature.

Evolving technologies such as blockchain and RFID are being tested across various food sectors, including seafood, and have the potential to complement the other analytical techniques described in this review. While these methods can provide rapid access to records of a product to the end user, there are still a few limitations which need to be addressed. While there have been a few implementations of these methods into small scale supply chains (Schröder, 2008), they remain relatively untested in the more complex supply chains which exist in the case of some seafood products. For instance, some seafood processors may use products from several countries; however, if only a few producers have implemented blockchain or RFID then then authenticity of the final processed fillets may be difficult to confirm. Additionally, because blockchain and RFID are essentially bookkeeping methods, provenance determination methods need to be available to ensure that all partners in a supply chain are held responsible for the input of data. The XRF through Itrax to use the elemental composition of the samples to determine the provenance of samples holds promise (Gopi et al., 2019a; 2019b). While it may not provide the quantified values of elements, for the purpose of determining provenance alone this is not a disadvantage. However, the reasons for variations between geographic locations and production methods need to be determined before the method can be

**Table 1**
A summary of the advantages and disadvantages of each methodology examined in this review.

| Method | Advantage | Drawbacks | Turnaround time | Non-destructive sampling? |
|---|---|---|---|---|
| DNA profiling | • determine species<br>• distinguish between geographic locations<br>• DNA profiling is offered by many labs<br>• can be used on processed seafood<br>• relatively cost-effective | • cannot be used to distinguish between production methods<br>• incomplete DNA profiles available for cross referencing<br>• different labs use different techniques | Relatively rapid | No |
| Fatty acid profiling | • can be used to distinguish between production methods and geographic origins<br>• relatively cost-effective | • samples require extensive preparation<br>• seasonal changes in fatty acid composition unclear at the moment | Relatively rapid | No |
| ICP – MS/AES/OES | • can be used to distinguish between production methods and geographic locations<br>• relatively cost-effective depending on the number of elements | • each element requires different preparation<br>• the elements necessary to distinguish between production methods and geographic locations differ according to species<br>• elemental composition can potentially be manipulated to have the same composition as a genuine product by fraudulent producers<br>• interpretation of results can vary according to type of tissue being analysed | Relatively rapid | No |
| SIA | • can be used to determine both production methods and geographic locations<br>• relatively cost-effective | • certain geographic locations and production methods may overlap with each other<br>• isotopic composition can potentially be manipulated through feed and environmental conditions | Slower than other methods | No |
| Blockchain | • can be used to determine species, production methods and geographic locations<br>• relatively easy to implement | • still untested in complex supply chains like seafood | Almost instantaneous | Yes |
| RFID | • can store species, production and location information<br>• has been utilised previously with seafood and has been shown as a cost-effective method | • difficult to implement in processed fillets of fish | Almost instantaneous | Yes |
| XRF through Itrax | • can distinguish between production methods and geographic locations<br>• relatively cost-effective for processing large batches of samples | • does not provide quantitative values of elements like ICP-MS/AES/OES<br>• elemental composition can potentially be manipulated to have the same composition as a genuine product by fraudulent producers | Relatively rapid | Yes |

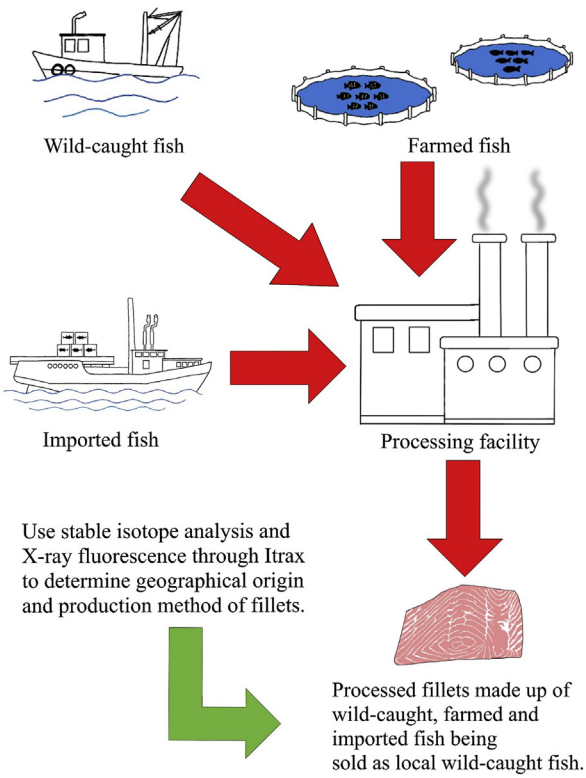


**Fig. 3.** An example of seafood fraud occurring, where the primary processor has substituted local wild-caught seafood with farmed and imported seafood.

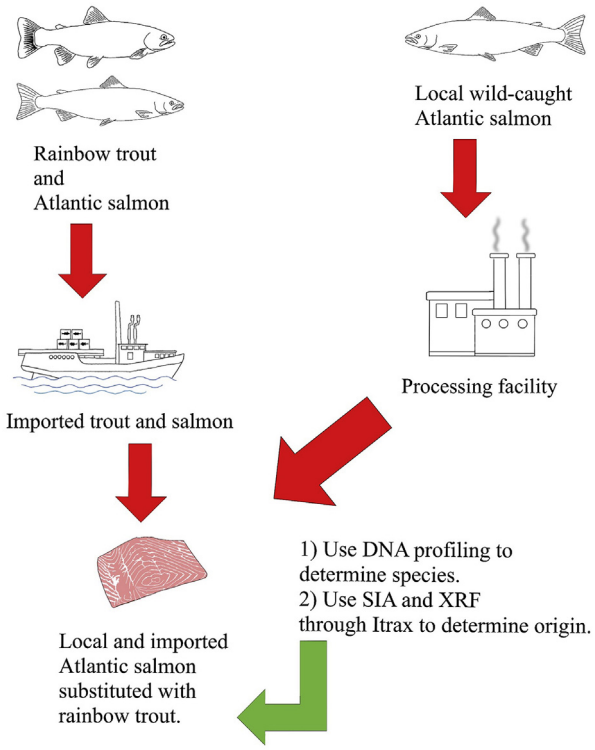


**Fig. 4.** Another example of seafood fraud occurring, where a fraudulent seller has substituted local wild-caught Atlantic salmon with imported rainbow trout and Atlantic salmon.

widely recommended for the purpose of seafood provenance determination.

Determining the provenance of seafood becomes difficult as seafood fraud becomes increasingly complex. There are several opportunities along the seafood supply chain for fraudulent businesses to substitute products. For instance, during primary processing there is an opportunity to add products from multiple origins and different species into a single batch (Fig. 3). This will increase profit because imported products are often cheaper than local those sourced locally. In order to detect this type of food fraud a combination of methods should be used. For instance, by using SIA and XRF through Itrax, the geographic origin and production method of the fillets can be determined (Gopi et al., 2019b). Utilising multiple methods is recommended here due to the limitations of the current methods. Similarly, a species substitution is often carried out by fraudulent businesses in order to increase profit margins (Fig. 4). DNA profiling is the only current method that can detect species substitution; to determine origin a combination of current methods should be used. These are just a few examples of how seafood fraud can occur along the supply chain. However, tackling these issues require multiple methods moment and addressing some of the shortfalls of the current methods will allow for better accuracy in determining the provenance of seafood.

## 5. Conclusion

There are a number of methodologies available to regulatory bodies and the seafood industry to determine the provenance of seafood with varying degrees of accuracy. Additionally, new technologies that enable the consumer to track the source of their seafood are being developed and tested. DNA profiling can be utilised to detect food substitution as well as, to a lesser extent, to discriminate between geographic locations of seafood. However, without a complete database of DNA profiles of different species, it can be difficult to recommend this method for determining the provenance of seafood, although it remains the de-facto standard for detecting food fraud. Fatty acid profiling has proved useful in discriminating between the production methods of seafood as well as their geographic locations. The only major drawback of this method is the seasonal variability in the fatty acid composition of seafood. Without a thorough understanding of how this might affect the determination of provenance, it is difficult to recommend the use of fatty acid profiling as a standalone method. Elemental profiling using ICP-MS and ICP-AES has shown promise when it comes to determining the provenance of seafood. To further improve the method, the elements that are vital in discriminating between geographic locations and production methods need to be determined to reduce the processing time and improve throughput. Stable isotope analysis of carbon and nitrogen has proven effective in distinguishing the provenance of seafood when combined with other methodologies. In addition, new technologies and methodologies are being developed for the purpose of seafood provenance. Without additional tests, it is difficult to comment on the utility of these methods on a large scale to combat widespread problems. As mentioned previously, the supply chain for seafood is highly complex. We believe that a combination of different methodologies is ideal for seafood provenance. Using a combination of techniques has advantages such as being able to predict the source of origin with a higher degree of accuracy than a single methodology alone. Additionally, a specific model that is developed for determining the provenance of seafood will add to this and provide regulatory bodies with the tools and techniques necessary for seafood traceability. This can also be used for compliance testing of methods like blockchain and RFID to ensure that the details stored in the system and being relayed to consumers are accurate.

Overall, this is a pivotal time for seafood provenance research, as several methodologies are currently in development. The cost-effectiveness of these methodologies needs to be determined before they become a widespread and common method for seafood provenance. By determining the provenance of seafood, food fraud can be detected and prevented, strengthening market and customer confidence. Additionally, it will protect consumer health as offending products

which cause outbreaks of disease can be recalled more quickly. Furthermore, regulatory bodies can be assured that imported seafood is from certified sources allowing for imported seafood to meet the needs of consumers. Overall, food provenance is more important now than ever as food producers gear up to feed a growing global population.

## Declaration of interest

The authors of this paper do not have any financial or personal relationships with other people or organisations that could inappropriately influence this review. The authors are not employed by the seafood industry and do not have any financial interest in the industry.

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