Aquaculture 502 (2019) 56–62

Contents lists available at ScienceDirect

Aquaculture

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

Isotopic and elemental profiling to trace the geographic origins of farmed and wild-caught Asian seabass (*Lates calcarifer*)

Karthik Gopi[a], Debashish Mazumder[b,*], Jesmond Sammut[a], Neil Saintilan[c], Jagoda Crawford[b], Patricia Gadd[b]

[a] Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney 2052, Australia
[b] Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia
[c] Department of Environmental Sciences, Macquarie University, Sydney, NSW, Australia

ARTICLE INFO

Keywords:
Provenance
Origin
L. calcarifer
Isotope
Elemental analysis
Food fraud

ABSTRACT

Demand for seafood, farmed or wild-caught, is growing globally. Consequently, seafood provenance is increasingly important to regulatory bodies, market chain actors and consumers. The limitations of current seafood provenance methods can be overcome using complementary or standalone nuclear techniques. This study focuses on determining the production method and geographic origin of Asian seabass (*Lates calcarifer*) using Stable Isotope Analysis (SIA) and X-ray fluorescence (XRF) through Itrax. The data were analysed using three different statistical methods; univariate and multivariate analysis, randomForest and LDA. The SIA model had accuracy of 84% when distinguishing the production methods and geographic origin of the *L. calcarifer*. The model using elemental analysis from the XRF returned an accuracy of 72%, and a combined SIA and elemental model was 81% accurate in determining provenance. However, the SIA model had two incorrect predictions compared to one incorrect prediction in the elemental model, while the combined model had no incorrectly predicted samples. The results of this study highlight that a combination of both SIA and elemental profiling through Itrax is ideal for seafood provenance.

1. Introduction

Demand for seafood is increasing globally, with aquaculture now exceeding the supply of seafood from wild fisheries (Botsford et al., 1997; Kearney, 2010). Currently, seafood accounts for 6% of protein in human diets, and it is expected that aquaculture will continue to grow as a source of seafood due to supply by wild fisheries peaking (Henchion et al., 2017). Fish and other seafood products are considered a healthier source of protein, compared to white and red meat, due to its unsaturated-fat content and high levels of iodine, omega-3 fatty acids, and a range of essential micronutrients (Sioen et al., 2007; World Health Organization, 2003). With the advent of better and more efficient aquaculture technologies, seafood production has become a major livelihood. Nevertheless, wild-caught seafood is a high value commodity, and is often sold alongside farmed fish and other aquaculture products (Buck, 2007).

Global trade of seafood involves moving large quantities of raw and processed products across regions. This has led to governments regulating the trade of seafood for environmental, economic, human health and biosecurity reasons. Regulatory controls are often based on certification and traceability requirements along the market chain, largely to identify the source of seafood to minimise the transfer of disease-carrying products that can affect human health, and to prevent product substitution (European Union, 2002; Ulrich et al., 2015). Seafood may carry harmful pathogens that can spread disease to wild fisheries and aquaculture industries, or may be deemed contaminated in the presence of banned chemicals, antibiotics – often associated with aquaculture – or heavy metals from industrial pollution (Feldhusen, 2000; Fyfe and Millar, 2012).

Although seafood provenance is embedded in regulations and certification and traceability protocols (European Union, 2002), it can be expensive and cumbersome to undertake mainly because analytical methods, such as DNA profiling, have limitations. For example, DNA profiling has limited application because it is most effective at distinguishing between species, and to a lesser extent, some genetic variation within a species that might indicate origin (Carrera et al., 2000; McGinnity et al., 1997). In the case of aquaculture, farmed seafood may be produced in one country, but broodstock or fingerlings/post larvae may originate from another. Similarly, microsatellite marker technologies may have limited use in distinguishing between farmed and wild-

* Corresponding author.
E-mail address: debashish.mazumder@ansto.gov.au (D. Mazumder).

https://doi.org/10.1016/j.aquaculture.2018.12.012
Received 13 August 2018; Received in revised form 26 November 2018; Accepted 4 December 2018
Available online 05 December 2018

caught seafood if hatcheries source broodstock locally or from different regions, or are used for stock enhancement for the wild fishery. Some aquaculture practices also involve collection of wild seed that carry the same microsatellite markers as their wild-grown counterparts (Estoup et al., 1998). Studies have also shown that fatty acids may be used to distinguish the source or production method of seafood, but the method involves a labour-intensive process to prepare samples for analysis (Budge et al., 2002; Nemova et al., 2013; Ricardo et al., 2015).

The application of stable isotopes to seafood provenance is recent (Moreno-Rojas et al., 2008; Serrano et al., 2007), and can complement other analytical technologies or potentially be applied as an independent provenance tool (Zhang et al., 2017), if refined. The underlying premise is that farmed and wild-caught fish have different diet sources and are exposed to different environmental conditions that lead to distinguishable differences in their isotopic and elemental signatures (Carter et al., 2015; Fry, 2006; Gamboa-Delgado et al., 2014; Kim et al., 2015; Kling et al., 1992; Mazumder et al., 2016; Ortea and Gallardo, 2015; Turchini et al., 2009). Stable isotope analysis (SIA) has been used effectively in the provenance of red meat, dairy products and wine (Primrose et al., 2010). Similarly, the elemental assimilation of fish can be influenced by the quality of water and diet, climatic conditions, and the management practices utilised in aquaculture (Alasalvar et al., 2002; Roy and Lall, 2006; Yamashita et al., 2006). For instance, elements, such as hafnium, are commonly found in seawater and therefore will accumulate in fish that have been raised in estuaries or wild-caught in saltwater (Rickli et al., 2010). However, since wild-caught Asian sea bass (*Lates calcarifer*), also known as barramundi, have a lifecycle which involves migration between fresh and sea water, it is likely that the elemental composition of these individuals will vary quite significantly in comparison to their farmed counterparts (Russell and Garrett, 1985).

Seafood provenance research and practices typically focus on commercially-valuable species such as Atlantic salmon (*Salmo salar*) (Carter et al., 2015; Gamboa-Delgado et al., 2014; Kim et al., 2015; Ortea and Gallardo, 2015; Turchini et al., 2009). Examples of such research include the study conducted by Molkentin et al. (2007), which showed a significant difference in $\delta^{13}C$ values between farmed and wild-caught Atlantic salmon (*S.salar*). Other studies showed there may or may not be a significant enrichment of $\delta^{15}N$ values for wild-caught Asian sea bass (*L. calcarifer*) (Moreno-Rojas et al., 2008; Serrano et al., 2007). Studies which have used SIA to determine the geographic origin of samples found SIA alone was not always accurate for provenance testing (Carter et al., 2015; Turchini et al., 2009). Further studies are needed to investigate the application of SIA combined with other analytical techniques, such as elemental profiling, to assess if the approach can accurately determine the geographic origin and production method.

Asian sea bass (*L. calcarifer*), is an ideal species for aquaculture because it is adaptable to wide-ranging water salinity and turbidity. The species has been farmed in brackish water, fresh water, and under marine conditions using different farming systems, such as ponds, tanks and floating cages. Pond or net-cage culture is the preferred method of cultivating and harvesting Asian sea bass (Boonyaratpalin and Williams, 2002; Talpur and Ikhwanuddin, 2012). Provenance of Asian sea bass is important because the farmed and wild-caught products are exported, and substitution or mislabelling can occur during different stages of the market chain leading to 'food fraud', and also compromising biosecurity in importing countries (Spink and Moyer, 2011). Consumers also expect to know the origin and production methods due to concerns over food safety and a desire to purchase sustainably produced fish. While it can be argued that the genetic variability of farmed *L. calcarifer* is decreasing due to hatchery culture practices, it would still be difficult to determine the geographic origin of farmed species using DNA profiling (Frost et al., 2006).

This study used stable carbon and nitrogen isotope analyses, and elemental profiling using X-ray fluorescence (XRF) through Itrax, to determine the geographic origin and production method of *L. calcarifer*, an important seafood product. XRF using Itrax provides a rapid analysis of multiple elements with very low detection limits and requires minimal sample preparation. The advantage of using Itrax over conventional elemental profiling methods is that it can detect a large number of elements (30 +), including heavy metals and lighter elements (Gopi et al., 2019).

The aim of this study is to determine if the two analytical technologies can be used to achieve a higher level of prediction for provenance to meet the requirements of regulations, and to trace *L. calcarifer* products to their geographic origin. As isotopic values of fish are closely related to diet (Fry, 2006; Mazumder et al., 2018) and elemental compositions related to their diet and environment (Alasalvar et al., 2002; Roy and Lall, 2006; Yamashita et al., 2006), we assume isotopic and elemental compositions of *L. calcarifer* will change with aquaculture practices and location. The following hypotheses were tested: the stable isotope values and elemental composition of Asian sea bass (*L. calcarifer*) will vary significantly according to the production methods (farm vs. wild) and geographic origin; and this variation can be used to accurately predict provenance.

2. Materials and methods

2.1. Sample collection

This study used authenticated samples obtained from wholesale market through collaboration with industry and research partners in Australia and Asia. Thirty-eight dead *L. calcarifer* samples were used to represent farms and wild fisheries from two regions within Australia (Northern Territory and Queensland) and one from Asia (Malaysia) (Fig. 1).

Each region had seven replicates for each production method ($n = 7$) except for Malaysia, which had five ($n = 5$). The farmed samples used for analysis were randomly collected from different ponds at each farm. Because isotopic values change due to ontogenetic diet shifts (Hentschel, 1998; Winemiller, 1989), the size range was kept similar, ranging from 60 to 70 cm; farmed fish were of a similar age. The age of wild-caught specimens was not determined, thus, length was used as an indicator of similar age. In the current study, all samples were of table size (approximately 60 cm) and ready for distribution to the market.

2.2. Sample preparation

The samples were immediately frozen on collection and transported to the research facilities. The samples from Asia were processed by partner-agency researchers at their own facility. Once they reached the laboratory, all samples were thawed and washed with de-ionised water before sample preparation for analysis. The fish samples were then descaled and cleaned with deionised water. A 5 cm^2 sample of tissue was removed from the dorsum of the *L. calcarifer* samples. Oven-dried dorsum samples, both Asian and Australian, were then transported to the Australian Nuclear Science and Technology Organisation (ANSTO) for isotopic and elemental analysis. All samples were checked to ensure that they were completely dry and then homogenised into a fine powder using a mortar and pestle (Kinney et al., 2011). The mortar and pestle were cleaned with ethanol between each sample to avoid cross-contamination. The fine powder was then stored in labelled airtight test tubes until they were used for stable isotope analysis and elemental profiling. To ensure consistency, each individual sample was sub-divided and used for both SIA and elemental profiling.

2.3. Stable isotope analysis

The isotopic analysis was conducted at ANSTO in Sydney, Australia, using a continuous-flow isotope ratio mass spectrometer (CF-IRMS), model Delta V Plus (Thermo Scientific Corporation, U.S.A.), interfaced with an elemental analyser (Thermo Fisher Flash 2000 HT EA, Thermo

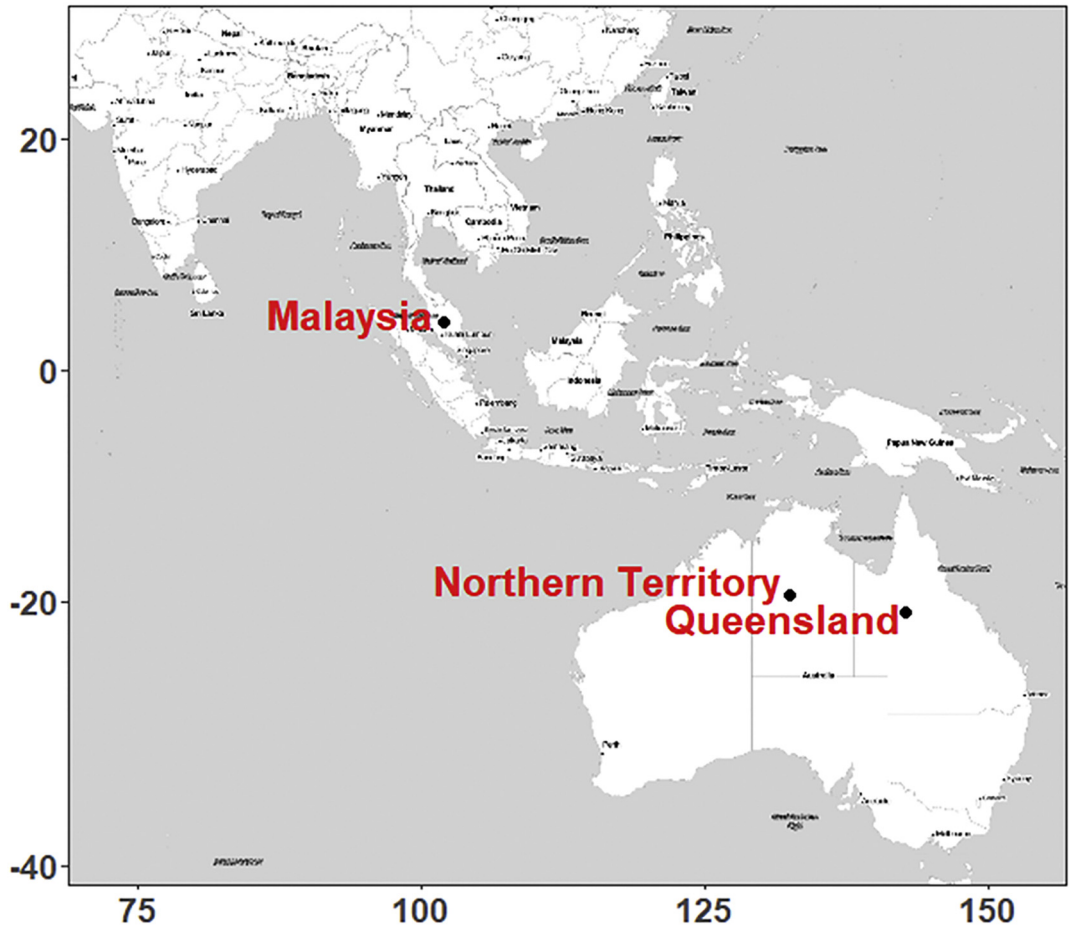

Fig. 1. Sample collection regions in Asia and in Australian states.

Electron Corporation, U.S.A.). Around 0.15 mg of each powdered sample was loaded into tin capsules and compacted manually to remove air spaces and then processed through CF-IRMS. All isotopic data were reported relative to IAEA (International Atomic Energy Agency) secondary standards, and certified relative to air for nitrogen, and Vienna-PeeDee Belemnite (VPDB) for carbon. Normalisation of the data was done using a two-point calibration, using standards (Chitin and Caesin Sodium Salt from Bovine Milk) which bracket the samples. Every run of the samples included both standards as quality control references. Results were accurate to 1% for both C% and N%, and ± 0.3 parts per thousand (‰) for $\delta^{13}C$ and $\delta^{15}N$. They were reported in delta (δ) units in parts per thousand (‰) determined by the formula:

$$X\,(‰) = \left(\frac{Rsample}{Rstandard} - 1\right) \times 1000$$

As lipid content in muscle can affect the $\delta^{13}C$ values of finfish (Sotiropoulos et al., 2004), the formula from Post et al. (2007) was used to mathematically correct for lipids if the C:N ratio was > 3.5:

$$\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}\,N$$

2.4. Elemental analysis

The procedure detailed by Gadd et al. (2018) was followed for elemental analysis. Around two grams of each powdered sample was attached to double-sided tape fixed to a clear acrylic sheet, and cleaned with ethanol. The samples were transferred to the tape with a spatula and flattened to around two centimetres in length using a paint scraper to ensure that the scan surfaces were uniform. The tools were cleaned with ethanol between samples to avoid cross-contamination. The powdered samples were then analysed using XRF spectrometry on the Itrax high-resolution core scanner at ANSTO (Croudace et al., 2006). The output spectra were fit to the model spectra using Q-Spec 8.6.0, which also accounted for elemental inferences and sum peaks. The XRF, through Itrax, determined the relative abundance of 31 different elements (Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Y, Zr, Cd, Sn, Sb, Nd, Hf, Pb, Bi, At, and U) present in the samples.

2.5. Data analyses

Three different statistical and ordination methods were used in this study to ensure that the results were accurate and consistent; they were: 1) univariate (ANOVA) and multivariate analysis (Principal Component Analysis (PCA)) in RStudio (R Core Team, 2018); 2) Linear Discriminant Analysis (LDA), a package in RStudio which can discriminate between multiple factors, and is utilised in provenance work (Natusch et al., 2017; Venables and Ripley, 2013); and, 3) randomForest, which is also an R package that shows promise in discriminating between factors (Liaw and Wiener, 2002).

LDA is a widely used dimensionality reduction technique (Sharma and Paliwal, 2015) and assumes normal distribution of data. The R package randomForest uses many classifiers (trees in this case, of which 500 were used), and does not assume any formal data distribution, to increase predictive performance (Liaw and Wiener, 2002). These trees were aggregated and used to distinguish between the chosen factors.

3. Results

Stable carbon and nitrogen isotope values differed significantly

Table 1
The ANOVA results of stable isotope values for *L. calcarifer* from the same geographic origin.

Lates calcarifer	Stable isotope	*F statistic*	Significance
Malaysia: farm vs. wild	$\delta^{13}C$	88.92	$p < .05$
	$\delta^{15}N$	63.08	$p < .05$
Queensland: farm vs. wild	$\delta^{13}C$	13.06	$p < .05$
	$\delta^{15}N$	193.9	$p < .05$
Northern Territory: farm vs. wild	$\delta^{13}C$	0.546	$p > .05$*
	$\delta^{15}N$	43.3	$p < .05$

* Indicates no significant difference.

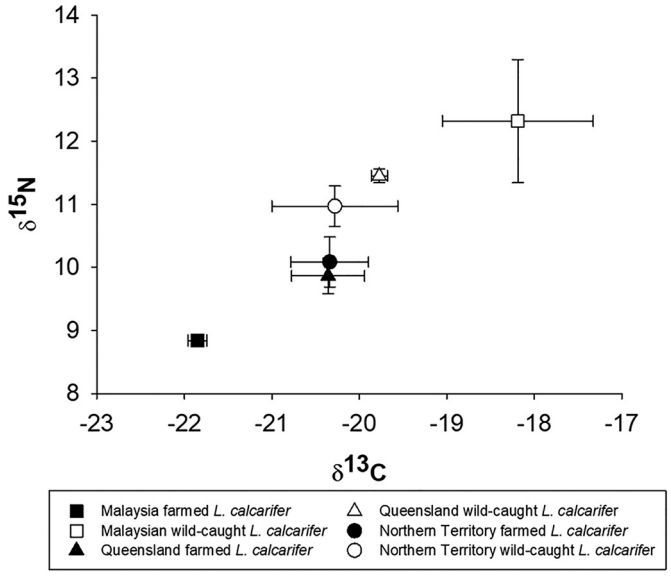

Fig. 2. Stable isotope biplot (mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values) of *L. calcarifer* differentiating farmed (filled in symbols) and wild-caught (empty symbols) samples. Samples were collected from 3 different geographic origins across Australia and Asia.

(Table 1) between farmed and wild-caught *L. calcarifer,* and the wild-caught fish samples were enriched in $\delta^{15}N$ (Fig. 2). The PCA of the SIA and elemental data combined together showed a difference between the farmed and wild-caught samples in most cases (Fig. 3). However, the farmed fish samples from Northern Territory had some overlap with the farmed fish samples from Queensland.

The SIA data showed that all 3 geographic origins were distinguishable through the $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 2). The PCA of the combined SIA and elemental abundance showed a clear distinction between all geographic origins of *L. calcarifer*.

The different datasets were used to create three separate models using randomForest and LDA. The randomForest had an accuracy of 84% for determining the production methods (farm vs. wild-caught) and geographic origin using SIA, while the accuracy of the analysis decreased to 72% when using elemental analysis. Combining the SIA and elemental datasets resulted in an accuracy of 81% for randomForest. However, when the different models were tested using a test dataset, the SIA model had two incorrect predictions out of six, while the elemental model only had one incorrect prediction out of six. The combined model had no incorrect predictions. The models built using LDA had an accuracy of 88% for isotopic data, 22% for elemental data and 9% for the combined dataset. When the prediction accuracy of the models were tested, the isotopic model, as well as the elemental model, predicted five out of six variables incorrectly, while the combined dataset had four incorrect predictions out of six.

4. Discussion

4.1. Production methods

Generally, isotopic values of consumer tissue are influenced by their diet (Carter et al., 2015; Mazumder et al., 2018), and the overlap between the Northern Territory and Queensland farmed fish samples are likely to be due to similar diets being used at the farms. Previous studies on finfish provenance show that both $\delta^{13}C$ and $\delta^{15}N$ values can be important for discriminating between varying production methods (Molkentin et al., 2007; Moreno-Rojas et al., 2008). Molkentin et al. (2007) showed that $\delta^{13}C$ values of Atlantic salmon (*S. salar*) differed significantly between farmed and wild individuals. Their study determined that the differences were due to the $\delta^{13}C$ values assimilated

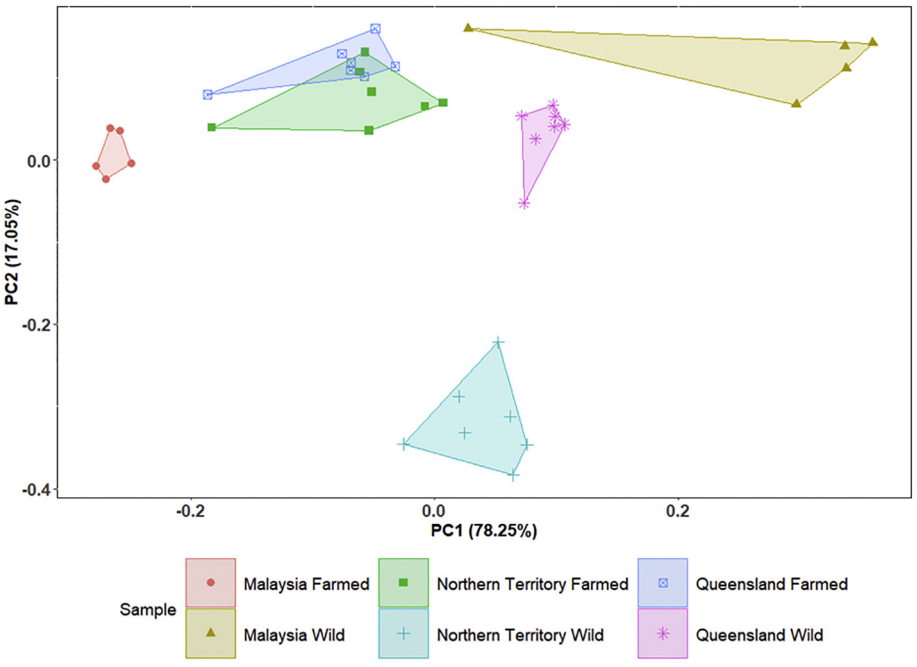

Fig. 3. PCA plot of the first two components showing the effectiveness of stable isotopes and relative elemental abundance for distinguishing the production method and geographic origin of *L. calcarifer*. The distance between groups indicates the differences between them. There is a clear difference between all farmed and wild-caught samples and most geographic origins, except for an overlap between the Northern Territory and Queensland farmed fish samples.

from either the C_3 or C_4 plants used in the feed. The present study found that $\delta^{13}C$ values contributed significantly to the difference between farmed and wild-caught *L. calcarifer* in the LDA. Generally, the wild caught samples of *L. calcarifer* were significantly enriched when compared to their farmed counterparts, with Northern Territory fish being the only exception. The depletion of $\delta^{13}C$ in the farmed samples is likely to be due to the use of terrestrial material, with a lower $\delta^{13}C$ content in the feed (Arechavala-Lopez et al., 2013). The present study found that the wild-caught *L. calcarifer* were significantly enriched in their $\delta^{15}N$ values when compared to their farmed counterparts. The difference in $\delta^{15}N$ values is likely due to the higher trophic- level natural food available to individuals in the wild. Similarly, the $\delta^{15}N$ values can be a reflection of the nitrogen sources used in the feed of the farmed *L. calcarifer* (Arechavala-Lopez et al., 2013). In addition, the $\delta^{15}N$ values are also used as an indicator of anthropogenic pollution in aquatic and estuarine systems. Typically organisms in systems polluted by anthropogenic sources have a higher $\delta^{15}N$ value (Lake et al., 2001). As the $\delta^{15}N$ values of the samples are relatively low, it can be assumed that it reflects the natural food rather than anthropogenic sources.

Elemental differences between farmed and wild-caught *L. calcarifer* can be attributed to the differences in diets and environmental conditions. Water and diet quality, climatic conditions, as well as management practices, influence the elemental assimilation of fish (Alasalvar et al., 2002; Roy and Lall, 2006; Yamashita et al., 2006). Anderson et al. (2010) conducted a similar study, using carbon and nitrogen stable isotopes, along with elemental abundance determined by ICP-MS, to distinguish between the production methods of three different types of salmon. Their study found that stable isotope and elemental abundance distinguished between the production methods of king salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), and Atlantic salmon (*S. salar*). The use of both SIA and elemental data in two separate models was recommended as it provides consistent results when distinguishing between farmed and wild-caught fish samples. The present study distinguishes itself from previous studies by using XRF through Itrax to determine the relative abundance of elements. The accuracy obtained by the present study (72%) was similar to the study by Anderson et al. (2010) who found > 75% accuracy, in spite of a lower number of replicates (five-seven samples compared to 64 for wild and 81 for farmed). The similarity in accuracy is presumably due to a larger number of elements (31) detected by the Itrax scanner as compared to the 19 used by Anderson et al. (2010) through ICP-MS.

4.2. Geographic origin

The LDA and randomForest models used in this study indicate that SIA can be used to determine the geographic origins of *L. calcarifer*. Kim et al. (2015) reported that the $\delta^{13}C$ values of mackerel (*Scomber japonicus*) and yellow croaker (*Larimichthys polyactis*) varied significantly between countries and with minimal variation within each country. For instance, their study reported that the samples obtained from four distinct sites in Korea had minimal variation. However, the present study found that the $\delta^{15}N$ values were significantly different among samples collected, even between the Australian states. The randomForest determined $\delta^{15}N$ as the most important variable when determining the geographic origin of samples. This result suggests that most geographic origins would have a unique isotopic signature (Carter et al., 2015; Fry, 2006; Gamboa-Delgado et al., 2014; Kim et al., 2015; Kling et al., 1992; Mazumder et al., 2016; Ortea and Gallardo, 2015; Turchini et al., 2009), which can be traced to authenticate the geographic origin of samples.

The elements that were detected by the XRF through Itrax are likely to be assimilated either from the environment or the diet of the individual (Anderson and Smith, 2005; Djedjibegovic et al., 2012; Hesslein et al., 1991; Lim et al., 2001; Rickli et al., 2010; Shim and Ng, 1988). Some elements, such as cadmium, could have potentially accumulated in the muscle tissue of individuals due to anthropogenic pollution, especially in estuaries (Thophon et al., 2003). As the environment and diets of the individuals will vary according to their production method, as well as geographic origins, it can be assumed that these contribute to the differences between the two factors. Determining the sources of these elements was not within the scope of this study, thus, it is recommended that their sources be studied further to underpin refinements in the application of elemental profiling as a seafood provenance tool. This can also be used to determine the effectiveness of specific elements as a tracer for the production methods and geographic origins of seafood.

The modelling of elemental data in this study was able to distinguish the geographic origins of *L. calcarifer*. The use of elemental data in provenance work is currently limited. Li et al. (2013) used Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) to determine the abundance of elements to distinguish the geographic origin of different Ictalurid catfish species. The eleven elements used in that study were sufficient to determine the geographic regions of the samples analysed. Similarly, Yamashita et al. (2006) showed that the elements derived from the Japanese eel (*Anguilla japonica*) were sufficient to distinguish their geographic origin. However, the advantage of the current study is the capacity of XRF, using the Itrax core scanner, to provide a larger dataset of elements, thus considerably improving precision when distinguishing the geographic origin of the samples.

4.3. Precision of models

The model created using stable isotope data had the highest accuracy; however, it also had the highest number of incorrectly predicted samples. This result was to be expected as previous literature suggested that SIA alone was not suited for seafood provenance (Carter et al., 2015; Turchini et al., 2009). The elemental data, which is a novel technique for seafood provenance, had only one incorrectly predicted sample even though it had a lower accuracy than the SIA model. Combining the two datasets results in no incorrectly predicted samples. This would suggest that a combination of both SIA and XRF through Itrax is suited for seafood provenance.

The discrepancy between the randomForest and LDA models suggest that the precision is also reliant on the model used. The relative inaccuracy of LDA could be due to the data not having a normal distribution and the model overfitting, which led to the incorrect classifications.

5. Conclusion

The present study has shown that the application of SIA and elemental profiling, through the Itrax core scanner, can determine the origin and production method of seabass collected from Australian and Asian sources. While the study was successful in achieving its aims, there are still limitations. For instance, the study did not account for seasonal variations of $\delta^{15}N$ (Sant'Ana et al., 2010) and was based on a limited sample size. Furthermore, the source of the elements should also be investigated in future studies along with more species and other factors, such as water quality and feed sources, to determine how these factors affect the models. Addressing these limitations in future studies will strengthen the accuracy of the model and allow for it to become a deterrent for seafood fraud.

Acknowledgements

The authors would like to thank the Australian Nuclear Science and Technology Organisation (ANSTO) for funding this research, Dr. Suzanne Hollins, Prof Marie-Claude and Prof Henk Heijnis, (ANSTO) for their support, and the Australian Institute of Nuclear Science and Engineering (AINSE) for funding to present this research at conferences. We extend our thanks to Mr Chris Calogeras, the Australian Barramundi Farmers Association, Simon Rowe from OceanWatch Australia, A/Prof

S M Nurul Amin (UPM, Malaysia) and Sydney Fish Market for their help with sample collection. A special thank you to Jennifer van Holst and Barbora Gallagher (ANSTO) for helping to process and analyse the samples for stable isotopes.

References

Alasalvar, C., Taylor, K.D.A., Zubcov, E., Shahidi, F., Alexis, M., 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chem. 79, 145–150.

Anderson, K.A., Smith, B.W., 2005. Use of chemical profiling to differentiate geographic growing origin of raw pistachios. J. Agric. Food Chem. 53, 410–418.

Anderson, K.A., Hobbie, K.A., Smith, B.W., 2010. Chemical profiling with modeling differentiates wild and farm-raised salmon. J. Agric. Food Chem. 58, 11768–11774.

Arechavala-Lopez, P., Fernandez-Jover, D., Black, K.D., Ladoukakis, E., Bayle-Sempere, J.T., Sanchez-Jerez, P., Dempster, T., 2013. Differentiating the wild or farmed origin of Mediterranean fish: a review of tools for sea bream and sea bass. Rev. Aquac. 5, 137–157.

Boonyaratpalin, M., Williams, K., 2002. Asian Sea Bass, *Lates calcarifer*. Nutrient Requirements and Feeding of Finfish for Aquaculture. pp. 40–50.

Botsford, L.W., Castilla, J.C., Peterson, C.H., 1997. The Management of Fisheries and Marine Ecosystems. Science 277, 509–515.

Buck, E.H., 2007. Seafood Marketing: Combating Fraud and Deception. Congressional Research Service, Library of Congress.

Budge, S.M., Iverson, S.J., Bowen, W.D., Ackman, R.G., 2002. Among-and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 59, 886–898.

Carrera, E., García, T., Céspedes, A., González, I., Fernández, A., Asensio, L.M., Hernández, P.E., Martín, R., 2000. Identification of smoked Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) using PCR-restriction fragment length polymorphism of the p53 gene. J. AOAC Int. 83, 341–346.

Carter, J.F., Tinggi, U., Yang, X., Fry, B., 2015. Stable isotope and trace metal compositions of Australian prawns as a guide to authenticity and wholesomeness. Food Chem. 170, 241–248.

Core Team, R., 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.

Croudace, I.W., Rindby, A., Rothwell, R.G., 2006. ITRAX: description and evaluation of a new multi-function X-ray core scanner. Geol. Soc. Lond., Spec. Publ. 267, 51–63.

Djedjibegovic, J., Larssen, T., Skrbo, A., Marjanović, A., Sober, M., 2012. Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS). Food Chem. 131, 469–476.

Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J.M., Adriamanga, M., Guyomard, R., 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). Mol. Ecol. 7, 339–353.

European Union, 2002. Regulation (EC) no 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Off. J. Eur. Communities 45, 1–24.

Feldhusen, F., 2000. The role of seafood in bacterialfoodborne diseases. Microbes Infect. 2, 1651–1660.

Frost, L.A., Evans, B.S., Jerry, D.R., 2006. Loss of genetic diversity due to hatchery culture practices in barramundi (*Lates calcarifer*). Aquaculture 261, 1056–1064.

Fry, B., 2006. Introduction. In: Fry, B. (Ed.), Stable Isotope Ecology. Springer New York, New York, NY, pp. 1–20.

Fyfe, M., Millar, R., 2012. Alarm at Antibiotics in Fish Imports 2012. The Sydney Morning Herald, Sydney.

Gadd, P., Gopi, K., Sammut, J., Saintilan, N., Crawford, J., Mazumder, D., 2018. Itrax micro X-ray fluorescence (μXRF) for soft biological tissues. Methods 5, 1267–1271.

Gamboa-Delgado, J., Molina-Poveda, C., Godínez-Siordia, D.E., Villarreal-Cavazos, D., Ricque-Marie, D., Cruz-Suárez, L.E., 2014. Application of stable isotope analysis to differentiate shrimp extracted by industrial fishing or produced through aquaculture practices. Can. J. Fish. Aquat. Sci. 71, 1520–1528.

Gopi, K., Mazumder, D., Sammut, J., Saintilan, N., Crawford, J., Gadd, P., 2019. Combined use of stable isotope analysis and elemental profiling to determine provenance of black tiger prawns (*Penaeus monodon*). Food Control 95, 242–248.

Henchion, M., Hayes, M., Mullen, A.M., Fenelon, M., Tiwari, B., 2017. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods 6, 53.

Hentschel, B.T., 1998. Intraspecific variations in δ13C indicate ontogenetic diet changes in deposit-feeding polychaetes. Ecology 79, 1357–1370.

Hesslein, R.H., Capel, M.J., Fox, D.E., Hallard, K.A., 1991. Stable Isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the Lower Mackenzie River Basin, Canada. Can. J. Fish. Aquat. Sci. 48, 2258–2265.

Kearney, J., 2010. Food consumption trends and drivers. Philos. Transac. R. Soc. B 365, 2793.

Kim, H., Kumar, K.S., Shin, K.-H., 2015. Applicability of stable C and N isotope analysis in inferring the geographical origin and authentication of commercial fish (Mackerel, Yellow Croaker and Pollock). Food Chem. 172, 523–527.

Kinney, M.J., Hussey, N.E., Fisk, A.T., Tobin, A.J., CAJMEPS Simpfendorfer, 2011. Communal or Competitive? Stable Isotope Analysis Provides Evidence of Resource Partitioning within a Communal Shark Nursery. Vol. 439. pp. 263–276.

Kling, G.W., Fry, B., O'Brien, W.J., 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology 73, 561–566.

Lake, J.L., McKinney, R.A., Osterman, F.A., Pruell, R.J., Kiddon, J., Ryba, S.A., Libby, A.D., 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. Can. J. Fish. Aquat. Sci. 58, 870–878.

Li, L., Boyd, C.E., Odom, J., Dong, S., 2013. Identification of ictalurid catfish fillets to rearing location using elemental profiling. J. World Aquacult. Soc. 44, 405–414.

Liaw, A., Wiener, M., 2002. Classification and regression by random forest. R News 2, 18–22.

Lim, C., Klesius, P.H., Shoemaker, C.A., 2001. Dietary iron and fish health. Nutr. Fish Health 189–199.

Mazumder, D., Wen, L., Johansen, M.P., Kobayashi, T., Saintilan, N., 2016. Inherent variation in carbon and nitrogen isotopic assimilation in the freshwater macro-invertebrate *Cherax destructor*. Mar. Freshw. Res. 67, 1928–1937.

Mazumder, D., Johansen, M.P., Fry, B., Davis, E.J.M., Research, F., 2018. Muscle and Carapace Tissue–diet Isotope Discrimination Factors for the Freshwater Crayfish *Cherax destructor*. vol. 69. pp. 56–65.

McGinnity, P., Stone, C., Taggart, J.B., Cooke, D., Cotter, D., Hynes, R., McCamley, C., Cross, T., Ferguson, A., 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES J. Mar. Sci. 54, 998–1008.

Molkentin, J., Meisel, H., Lehmann, I., Rehbein, H., 2007. Identification of organically farmed Atlantic salmon by analysis of stable isotopes and fatty acids. Eur. Food Res. Technol. 224, 535–543.

Moreno-Rojas, J.M., Tulli, F., Messina, M., Tibaldi, E., Guillou, C., 2008. Stable isotope ratio analysis as a tool to discriminate between rainbow trout (*O. mykiss*) fed diets based on plant or fish-meal proteins. Rapid Commun. Mass Spectrom. 22, 3706–3710.

Natusch, D.J., Carter, J.F., Aust, P.W., Van Tri, N., Tinggi, U., Riyanto, A., Lyons, J.A., 2017. Serpent's source: determining the source and geographic origin of traded python skins using isotopic and elemental markers. Biol. Conserv. 209, 406–414.

Nemova, N.N., Fokina, N.N., Nefedova, Z.A., Ruokolainen, T.R., Bakhmet, I.N., 2013. Modifications of gill lipid composition in littoral and cultured blue mussels *Mytilus edulis* L. under the influence of ambient salinity. Polar Rec. 49, 272–277.

Ortea, I., Gallardo, J.M., 2015. Investigation of production method, geographical origin and species authentication in commercially relevant shrimps using stable isotope ratio and/or multi-element analyses combined with chemometrics: an exploratory analysis. Food Chem. 170, 145–153.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montaña, C.G., Rosenheim, J., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189.

Primrose, S., Woolfe, M., Rollinson, S., 2010. Food forensics: methods for determining the authenticity of foodstuffs. Trends Food Sci. Technol. 21, 582–590.

Ricardo, F., Pimentel, T., Moreira, A.S.P., Rey, F., Coimbra, M.A., Rosário Domingues, M., Domingues, P., Costa Leal, M., Calado, R., 2015. Potential use of fatty acid profiles of the adductor muscle of cockles (*Cerastoderma edule*) for traceability of collection site. Sci. Rep. 5, 11125.

Rickli, J., Frank, M., Baker, A.R., Aciego, S., de Souza, G., Georg, R.B., Halliday, A.N., 2010. Hafnium and neodymium isotopes in surface waters of the eastern Atlantic Ocean: implications for sources and inputs of trace metals to the ocean. Geochim. Cosmochim. Acta 74, 540–557.

Roy, P.K., Lall, S.P., 2006. Mineral nutrition of haddock *Melanogrammus aeglefinus* (L.): a comparison of wild and cultured stock. J. Fish Biol. 68, 1460–1472.

Russell, D., Garrett, R., 1985. Early life history of barramundi, *Lates calcarifer* (Bloch), in north-eastern Queensland. Mar. Freshw. Res. 36, 191–201.

Sant'Ana, L.S., Ducatti, C., Ramires, D.G., 2010. Seasonal variations in chemical composition and stable isotopes of farmed and wild Brazilian freshwater fish. Food Chem. 122, 74–77.

Serrano, R., Blanes, M.A., Orero, L., 2007. Stable isotope determination in wild and farmed gilthead sea bream (*Sparus aurata*) tissues from the western Mediterranean. Chemosphere 69, 1075–1080.

Sharma, A., Paliwal, K.K., 2015. Linear discriminant analysis for the small sample size problem: an overview. Int. J. Mach. Learn. Cybern. 6, 443–454.

Shim, K.F., Ng, S.H., 1988. Magnesium requirement of the guppy (*Poecilia reticulata*, Peters). Aquaculture 73, 131–141.

Sioen, I., Matthys, C., De Backer, G., Van Camp, J., Henauw, S.D., 2007. Importance of seafood as nutrient source in the diet of Belgian adolescents. J. Hum. Nutr. Diet. 20, 580–589.

Sotiropoulos, M.A., Tonn, W.M., Wassenaar, L.I., 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. Ecol. Freshw. Fish 13, 155–160.

Spink, J., Moyer, D.C., 2011. Defining the public health threat of food fraud. J. Food Sci. 76, R157–R163.

Talpur, A.D., Ikhwanuddin, M., 2012. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against Vibrio harveyi infection in Asian sea bass, *Lates calcarifer* (Bloch). Aquaculture 364–365, 6–12.

Thophon, S., Kruatrachue, M., Upatham, E.S., Pokethitiyook, P., Sahaphong, S., Jaritkhuan, S., 2003. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environ. Pollut. 121, 307–320.

Turchini, G.M., Quinn, G.P., Jones, P.L., Palmeri, G., Gooley, G., 2009. Traceability and discrimination among differently farmed fish: a case study on Australian Murray cod. J. Agric. Food Chem. 57, 274.

Ulrich, R.M., John, D.E., Barton, G.W., Hendrick, G.S., Fries, D.P., Paul, J.H., 2015. A

handheld sensor assay for the identification of grouper as a safeguard against seafood mislabeling fraud. Food Control 53, 81–90.
Venables, W.N., Ripley, B.D., 2013. Modern Applied Statistics with S-PLUS. Springer Science & Business Media.
Winemiller, K.O., 1989. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan ilanos. Environ. Biol. Fish 26, 177–199.
World Health Organization, 2003. Food Based Dietary Guidelines in the WHO European Region. WHO, Copenhagen, Denmark.
Yamashita, Y., Omura, Y., Okazaki, E., 2006. Distinct regional profiles of trace element content in muscle of Japanese eel *Anguilla japonica* from Japan, Taiwan, and China. Fish. Sci. 72, 1109–1113.
Zhang, X., Liu, Y., Li, Y., Zhao, X., 2017. Identification of the geographical origins of sea cucumber (*Apostichopus japonicus*) in northern China by using stable isotope ratios and fatty acid profiles. Food Chem. 218, 269–276.