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Combined use of stable isotope analysis and elemental profiling to determine provenance of black tiger prawns (*Penaeus monodon*)

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ABSTRACT

Global demand for seafood is rising, with a commensurate increase in supply from farmed and wild-caught products. Determining seafood provenance is important to reduce food fraud, and food safety and biosecurity risks. DNA and fatty acid profiling cannot independently distinguish between farmed, wild-caught and geographic origins of seafood. This study applied stable isotope analysis (SIA) and X-ray fluorescence (XRF), using Itrax, to test their effectiveness as tools to distinguish the origin and production methods of black tiger prawns (*Penaeus monodon*) from a range of Asia-Pacific locations. Isotopic and elemental data (31 elements) were analysed using multivariate methods, linear discriminant analysis (LDA), and randomForest. LDA and randomForest had consistent results: XRF effectively distinguished the production method and geographic origin of *P. monodon* (up to 100% accuracy), while SIA had a lower accuracy (up to 95% accuracy). However, SIA and XRF are effective complementary methods for determining provenance of black tiger prawns.

1. Introduction

Black tiger prawn (*Penaeus monodon*) is a commercially-important species and is widely exported as a raw or processed product. Prawn farming has advanced rapidly in response to increasing demand from the global market that cannot be met from the capture fishery. However, expansion and intensification of prawn farming has raised concerns over environmental impacts and food quality (Waite et al., 2014). Tiger prawn production has been under scrutiny due to concerns over accumulation of toxins, hormones and antibiotics and, consequently, risks to human health (Mansfield, 2011). Black tiger prawns, farmed under different environmental conditions and practices, assimilate minerals and pollutants from their surroundings (Anderson & Smith, 2005; Wang & Fisher, 1996). Globally, there have been increasing concerns over food safety, hygiene and authenticity of imported seafood, such as black tiger prawns (Furness & Osman, 2006; Ulrich et al., 2015), due to the presence of bacterial pathogens detected in imported seafood (Feldhusen, 2000). Importing seafood can also pose a risk to biosecurity and local seafood production through the introduction of pathogens that can trigger disease epidemics and pandemics. Furthermore, black tiger prawn, being a high value seafood product, can be illegally replaced by other, lower cost species, and fraudulently mislabelled, particularly when sold with the head and shell removed. These issues highlight the importance of seafood provenance because products are often traded across a wide geographic area.

Currently, provenance determination of seafood relies on several techniques with DNA profiling the most common method (Lees, Humber Institute of, & Fisheries, 2003). However, DNA profiling cannot identify production methods or origin because the differences in the DNA of farmed and wild-caught fish, and other seafood commodities, are unlikely to be significant (Carrera et al., 2000; McGinnity et al., 1997) unless there is a clear difference in the DNA profile due to genetic drift (Cross & Challanain, 1991; McGinnity et al., 1997; Scarano & Rao, 2014). Further, DNA profiling for geographic origin is confounded by the widespread export of fingerlings, post larvae of prawns (known as shrimp in some countries), and broodstock for aquaculture production. Clearly, there is a need for alternative or complementary methods to identify production sources and the geographic origin of seafood products.

Stable isotope analysis (SIA) has been used to authenticate seafood products based on differences in carbon and nitrogen isotope values in food sources (e.g., farmed and wild) which are ultimately reflected by the consumers (Carter, Tinggi, Yang, & Fry, 2015; Gamboa-Delgado et al., 2014; Gopi, Mazumder, Saintilan, & Sammut, 2018; Kim, Kumar,

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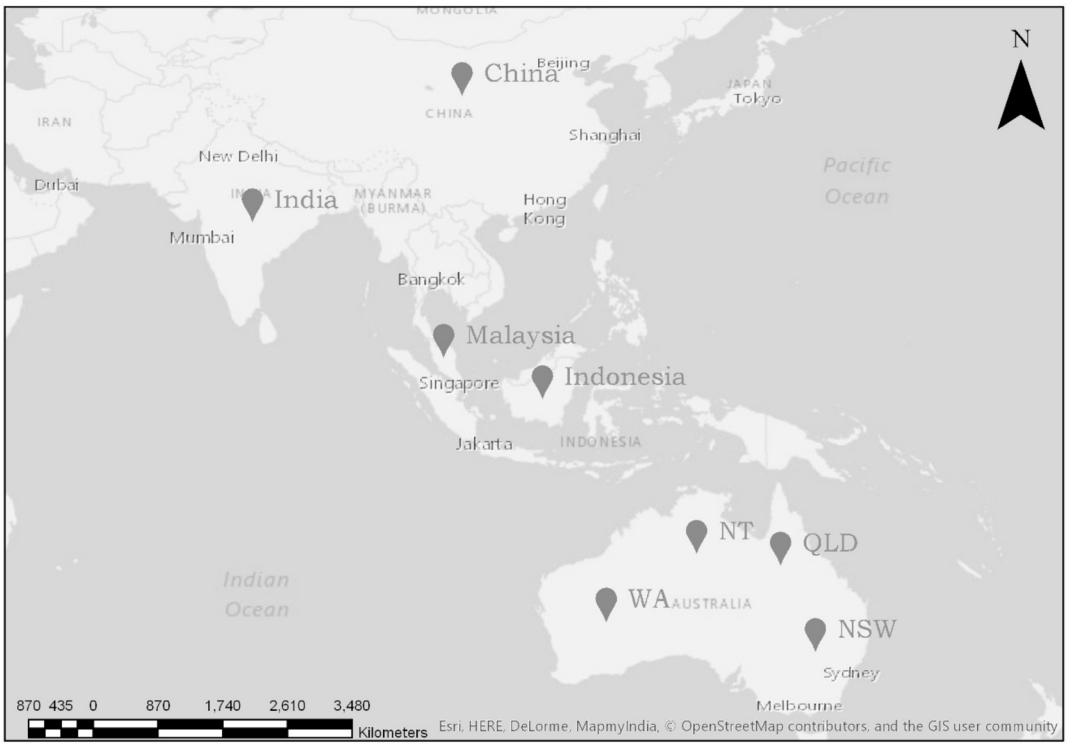

Fig. 1. Sample collection regions in Asia and Australia.

& Shin, 2015; Ortea & Gallardo, 2015; Turchini, Quinn, Jones, Palmeri, & Gooley, 2009).

However, stable isotope analysis does not always accurately determine the geographic origin of fish (Carter et al., 2015; Turchini et al., 2009) due to lack of sufficient isotopic enrichment between farmed and wild environments (Serrano, Blanes, & Orero, 2007). This suggests application of other techniques is needed in conjunction with SIA to increase resolution and predictability to accurately determine the geographic origin of products and their production methods (Gopi et al., 2018).

Elemental profiling has also been successfully used for provenance (Li et al., 2017). The validity of using elemental profiling to investigate the production method or the geographic origin of any fish is based on the notion that the mineral and trace metal composition of individuals reflects the source environment (Anderson & Smith, 2005) and feed (Reinfelder & Fisher, 1994). Li, Boyd, and Odom (2014) determined provenance of Pacific white shrimp (*Litopenaeus vannamei*) using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The study distinguished Pacific white shrimp from three distinct geographic origins using 20 elements. The main constraint of using ICP-AES is that samples are destroyed in the process thereby limiting re-use of samples for complementary analytical methods or re-analyses. By contrast, high-resolution micro-x-ray fluorescence (XRF), via an Itrax core scanner, is non-destructive. The Itrax core scanner is capable of generating micro-XRF and micro-radiographic images as fine as 0.2 mm in resolution (Croudace, Rindby, & Rothwell, 2006). However, elemental profiling through Itrax has yet to evolve as a tool for determining production methods and the geographic origin of seafood products.

This paper applied SIA, in conjunction with elemental profiling through high resolution XRF, using an Itrax core scanner, for the first time, to determine if tiger prawns originated from aquaculture (farmed) or were caught from the wild, and to identify geographic origin. The following hypotheses were tested using *P. monodon* commonly found in the Asia-Pacific region: 1) the stable isotope values and elemental composition of *P. monodon* will vary significantly according to the production methods (farmed vs wild-caught); 2) the stable isotopic values and elemental composition will vary significantly according to the geographic regions. A scoping study by Gopi et al. (2018) distinguished between farmed and wild-caught prawns (*P. monodon*) using SIA for 14 samples from one location in Australia. However, the study was limited to SIA only and the data analysis was restricted to Analysis of Variance (ANOVA). While that study demonstrated the feasibility of SIA in determining the production methods for prawns, it was limited to a small sample size and the impact of sampling from multiple locations was not explored. The present study used both SIA and elemental profiling, via Itrax, for 73 samples collected from various geographical locations to differentiate between production methods and to determine the geographical origin of prawns. Furthermore, we also determined the independent and combined use of these two analytical tools to determine provenance. In addition three statistical methods were used to analyse the data to compare their efficacy in relation to the provenance tools.

2. Materials and methods

2.1. Sample collection

The use of authentic samples is fundamental to seafood provenance research, and the only way to ensure sample authenticity is to collect them directly from their origin. In this study authenticity of the tested samples was ensured through collaboration with industry and research networks. A total of 73 *P. monodon* samples were collected, which included farmed and wild-caught samples from three Australian states (New South Wales, Queensland and Western Australia) and one territory (Northern Territory), and four Asian countries (China, India, Indonesia and Malaysia) (Fig. 1). Australia is a large continent, therefore samples were collected from different locations to account for bioregional variation (Barr & Possingham, 2013; Butler, Rees, Beesley, & Bax, 2010). *P. monodon* samples from Australia were collected with the help of the Australian Prawn Farmers Association (APFA). Asian prawn samples were collected through trusted research partners in

India, Indonesia, and Malaysia. The Chinese prawn samples were collected from Sydney Fish Market in New South Wales, Australia through OceanWatch Australia.

A previous study (Mazumder, Williams, Reid, Saintilan, and Szymczak (2008)) indicated that at least 5 samples are required to characterise SIA signatures of local populations of marine invertebrates. Seven replicated samples (n = 7) were used except for Malaysian samples which had five (n = 5) replicates. 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. *P. monodon* ranged from 10 to 14 cm were of a similar age, but 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 market size and ready for distribution.

2.2. Sample preparation

For this study, all the samples were immediately frozen on collection and transported to the research facilities. Once they reached the laboratory, all samples were thawed and washed with de-ionised water before sample preparation for analysis. The *P. monodon* samples had their exoskeleton, hindgut, and gonads removed before being cleaned with deionised water. A 5 cm^2 sample of abdominal tissue was removed from the ventral side of the *P. monodon* samples.

The tissue samples were placed in clean Petri dishes and oven-dried at ~60 °C for 48 h. The Asian samples were transported to the Australian Nuclear Science and Technology Organisation (ANSTO). All samples were checked to ensure that they were completely dry and then homogenised into a fine powder using a mortar and pestle (Kinney, Hussey, Fisk, Tobin, & Simpfendorfer, 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

For SIA, 0.15 mg of each powdered sample was loaded into tin capsules and compacted manually to remove air spaces (Kinney et al., 2011). 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 Electron Corporation, U.S.A.). All data were reported relative to IAEA (International Atomic Energy Agency) secondary standards, and were certified relative to air for nitrogen, and Vienna-PeeDee Belemnite (VPDB) for carbon. A two-point calibration was used to normalise the data, using standards (Chitin and Caesin Sodium Salt from Bovine Milk) which bracket the analysed samples. Both of these standards were used as quality control references and were included in every sample run. All 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 crustaceans (Bodin, Le Loc'h, & Hily, 2007; Stenroth et al., 2006) the formula from Post et al. (2007) was used to mathematically correct for lipids if the C:N ratio was greater than 3.5:

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

2.4. Elemental analysis

Around 2 g of each powdered sample was attached to a Perspex sheet using double-sided adhesive tape. The Perspex sheet was cleaned using ethanol, and the double-sided adhesive tape was fixed to the middle of the sheet. Afterwards, 2 cm intervals were measured along the tape and labelled with the sample number. Each sample was transferred to the tape with a 2-cm spacing between each, and flattened using a paint scraper to ensure that the scan surfaces were uniform. The spatula and scraper 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). Q-Spec 8.6.0 was used to fit the spectra to the model spectra, and to account 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 farmed and wild-caught samples from seven different bio-regions.

2.5. Data analyses

Three different statistical and ordination methods were used in this study to ensure that the results were accurate and consistent: 1) univariate (ANOVA) and multivariate analysis through the use of PRIMER V6 (Anderson, Gorley, & Clarke, 2008), commonly used to explore the similarities of stable isotopes and elements between farmed and wild-caught samples, and geographical origins; 2) LDA, a package in RStudio which can discriminate between multiple factors, and is utilised in provenance work (Natusch et al., 2017; Venables & Ripley, 2013); and, 3) randomForest, which is also an R package module that shows promise in discriminating between factors (Liaw & Wiener, 2002).

We compared the predictive ability of LDA (which assumes normality) and randomForest (in which no formal distribution is assumed). The stable isotope and elemental abundance dataset were separated into two groups: the training set, and the test set. The training set contained all but one replicate of each origin to develop the models. The test dataset was then used to test the accuracy of the models in predicting whether the samples were farmed or wild-caught, and to determine their geographic origin. In this study, the LDA function from the R package MASS was used (Venables & Ripley, 2013). Because there was a difference in values between the different elements, the values were scaled by subtracting the mean and dividing by the standard deviation before building the model.

The R package randomForest (Liaw & Wiener, 2002) is a type of "ensemble learning" method where many classifiers (trees in this case of which 500 were used) are generated. These trees are aggregated and used to distinguish between farmed and wild-caught samples, and the geographic origin of each species. In randomForest, each tree is constructed using a different bootstrap sample of the data, and in addition, each node is split using the best subset of predictor variables such as stable isotope data or elemental profiles. During the tree building phase, an error rate is calculated, which is the error in predicting the data that were not used in the bootstrap sample; this is referred to as the "out-of-bag" (OOB) error rate. The method employed by randomForest is recognized for its accuracy and its ability to deal with small sample sizes and high-dimensional spaces (Biau & Scornet, 2016). Here we investigated the impact of sample size on the OOB error rate by removing samples from each group. Using the ITRAX data resulted in an OOB error rate of 1.37, 3.23, 0, 2.50, 6.90 and 15.00%, when 7, 6, 5, 4, 3, 2 samples in each group were used, respectively. This indicates that for this data set ~5 samples from each group were sufficient. For LDA the accuracy in predicting the removed samples (using leave one out cross validation) was 98% for 7 and 6 samples in each group, 90% for 5 samples and then was reduced to 5% for the remaining.

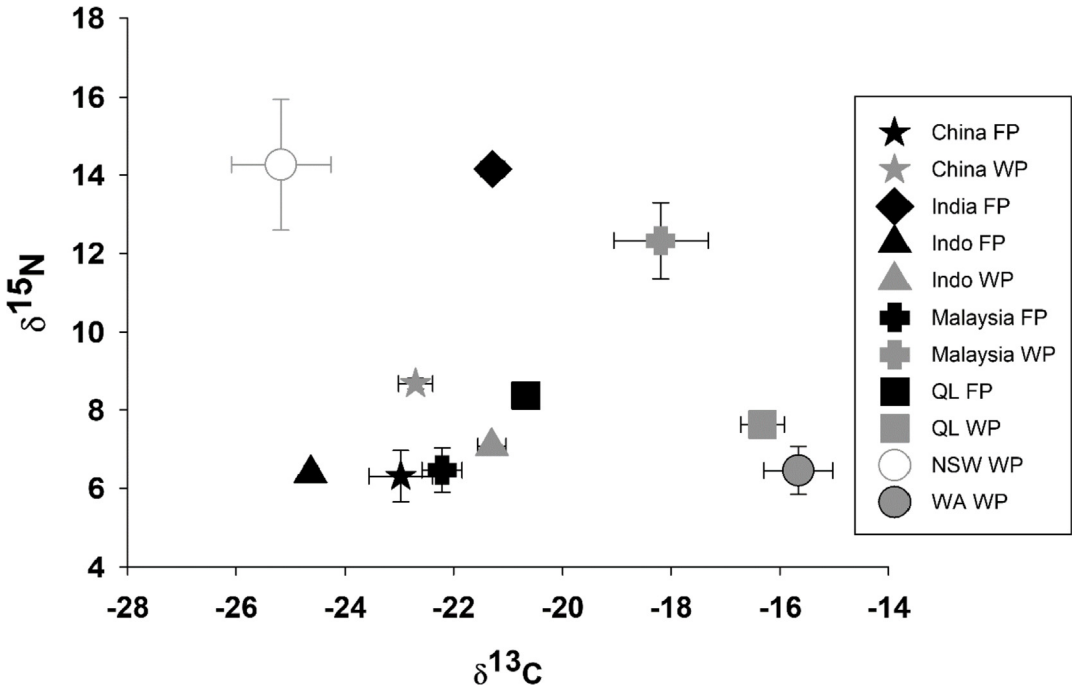

Fig. 2. Stable isotope biplot (mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values) of *P. monodon*, differentiating between farmed (in black) and wild-caught (in grey) samples. Samples were collected from 7 different geographical locations across Australia and Asia. Figure legend: FP = farmed, WP = wild-caught, Indo = Indonesia, QL = Queensland, NSW = New South Wales, and WA = Western Australia.

3. Results

The stable carbon and nitrogen bi-plot of *P. monodon* shows a clear distinction between the farmed and wild-caught samples from the same location (Fig. 2). The wild *P. monodon* generally had enriched $\delta^{13}C$ values compared to the farmed samples from the same region, except for China. Univariate analysis (ANOVA) confirmed that stable isotope values were significantly different between farmed and wild-caught *P. monodon* (Table 1).

The nMDS ordinations of stable isotopes (Fig. 3A) and elemental abundance (Fig. 3B) shows differences between farmed and wild-caught *P. monodon*. ANOSIM tests confirm that the difference was significant for SIA (ANOSIM p = 0.0005, Global R = 0.14) and for the relative elemental abundance (ANOSIM p = 0.007, Global R = 0.073) of *P. monodon*. Pooling the Asian and Australian samples together revealed a distinct difference between the two regions using elemental data (ANOSIM p = 0.0004). However, the SIA data revealed no significant differences between the two regions (ANOSIM p = 0.48).

For most cases, geographical locations were distinguishable using $\delta^{13}C$ and $\delta^{15}N$ values of *P. monodon* (Fig. 2). Out of the 7 locations, India, Indonesia, Queensland, New South Wales, and Western Australia were clearly distinguishable through the carbon and nitrogen bi-plot. However, both carbon and nitrogen stable isotope values of farmed Chinese and Malaysian samples overlapped. The ANOVA revealed a significant difference between the $\delta^{13}C$ values (p = 0.0319) but no significant difference in the $\delta^{15}N$ values (p = 0.473).

nMDS ordinations and ANOSIM of isotopic composition (Fig. 3C) and elemental abundance (Fig. 3D) of *P monodon* indicate a significant difference in their stable isotope values (ANOSIM p = 0.0001, Global R = 0.7) and their elemental abundances (ANOSIM p = 0.0001, Global R = 0.698), between all geographic locations.

The LDA (Fig. 4A) and randomForest discriminated between farmed and wild-caught *P. monodon*, as well as their geographic locations, with an accuracy of 90% and 95%, respectively, using stable isotopes. Using elemental abundance, the accuracy was increased to 100% for LDA (Fig. 4B) and 98% for randomForest. Further exploration of the data revealed that using only the essential elements (Mg, Cu, Ca, K, Fe, Se, and Zn) gave the analyses a similar accuracy when compared to using all 31 different elements. However, using only the most abundant variables led to a decreased accuracy (LDA: 94%; randomForest: 82%). Removing the elements contributing the least to the discrimination led to minimal changes to the accuracy (Table 2). Further exploration of the data by combining the SIA and Itrax dataset resulted in the same overall accuracy as the Itrax dataset, when all 31 elements were used.

Table 1
The ANOVA results for stable isotope values of *P. monodon*.

Penaeus monodon	Stable isotope	*F statistic*	Significance
China:farm vs.wild	$\delta^{13}C$	90.87	5.99E-07
	$\delta^{15}N$	0.839	0.378*
Indonesia:farm vs.wild	$\delta^{13}C$	927.1	9.88E-13
	$\delta^{15}N$	151.9	3.59E-08
Malaysia:farm vs.wild	$\delta^{13}C$	30.59	0.000553
	$\delta^{15}N$	237.4	3.13E-07
Queensland:farm vs.wild	$\delta^{13}C$	621.4	1.05E-11
	$\delta^{15}N$	44.49	2.29E-05

* indicates no significant difference.

4. Discussion

Given increasing concerns over the safety and authenticity of imported foods, and specifically the risks to the biosecurity of prawn aquaculture through the outbreak of diseases, the need for reliable and accurate methods of prawn provenance is clear. Previous studies distinguishing between farmed and wild-caught seafood, and their geographic origins, have often found conflicting results (Gamboa-Delgado et al., 2014; Li et al., 2014; Ortea & Gallardo, 2015). Li et al. (2014) had successfully discriminated between the geographic origin of prawns using elemental profiling. On the other hand, Ortea and Gallardo (2015) found that SIA was better suited to distinguish between production methods as well as the geographic origins of prawns, though their study used a limited range of elements. The current study has shown that SIA, and elemental profiling through Itrax, can successfully

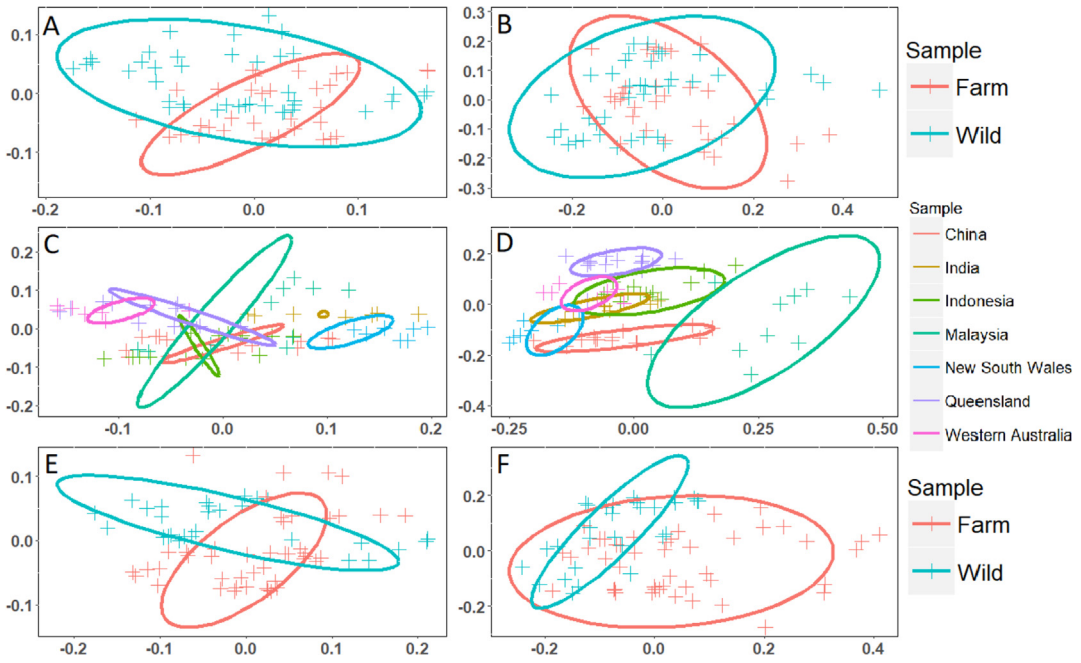

Fig. 3. nMDS ordinations for (A) SIA and (B) relative elemental abundances differentiating between farmed and wild-caught samples, and (C) SIA and (D) relative elemental abundance for distinguishing the geographic locations of *P. monodon* (E) SIA and (F) relative elemental abundance distinguishing between Australian and Asian *P. monodon*. Samples were obtained from 7 different geographical locations across Australia and Asia. Figure legend: QLD = Queensland, NSW = New South Wales, and WA = Western Australia.

distinguish between farmed and wild-caught samples of *P. monodon*. In the context of the present study, the geographic locations of *P. monodon* can also be determined using the two analytical methods.

The significant difference between the stable carbon and nitrogen values of farmed and wild-caught *P. monodon* demonstrates the efficacy of isotopic techniques for seafood provenance. The LDA and randomForest also effectively distinguished between production methods using stable isotope values with high accuracy (> 90%). This finding is consistent with the study by Gamboa-Delgado et al. (2014), and Ortea and Gallardo (2015), which determined that carbon and nitrogen stable isotopes are highly suitable for distinguishing between farmed and wild-caught prawn samples.

The enriched $\delta^{13}C$ and $\delta^{15}N$ values of the wild-caught *P. monodon* are linked to their diet and the higher trophic position they occupy in natural ecosystems (Gamboa-Delgado et al., 2014). Typically *P. monodon* diet contains crustaceans, molluscs, fish, polychaetes, echinodermata, debris, along with silt and sand particles (Marte, 1980). Because farms are a relatively closed system, and other food sources are purged in the pond preparation stage, the consistent supply of

Table 2
Summary of LDA and randomForest results for *P. monodon*.

Farm vs wild and geographical differences				
Species	Methods	Statistics used	Accuracy	Discriminant variable
P.monodon	SIA	randomForest	95%	$\delta^{15}N$
		LDA	90%	$\delta^{13}C$
	Itrax-31 elements	randomForest	98%	Copper
		LDA	100%	Phosphorus
	Itrax-Low contributions removed	randomForest	95%	Copper
		LDA	100%	Iron
	Itrax-7 essential elements	randomForest	97%	Copper
		LDA	98%	Potassium
	Itrax-Most abundant elaments	randomForest	82%	Potassium
		LDA	94%	Chlorine

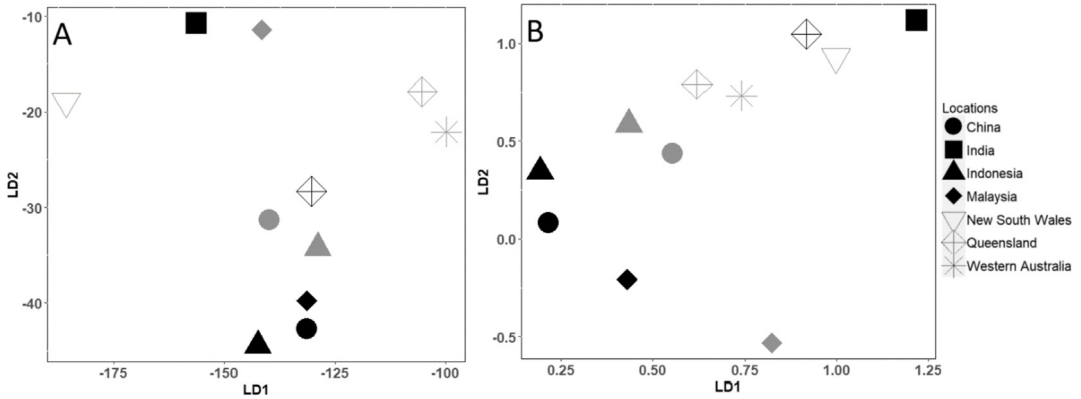

Fig. 4. LDA plot demonstrating the separation in both farmed (in black) and wild-caught (in grey) samples, as well as geography using (A) $\delta^{13}C$ and $\delta^{15}N$ stable isotope values and C:N ratios and (B) elemental compositions of *P. monodon*.

formulated feed depleted in $\delta^{13}C$ leads to the farmed samples being less enriched than wild-caught samples (Gamboa-Delgado & Le Vay, 2009; Gamboa-Delgado et al., 2014; Mazumder, Wen, Johansen, Kobayashi, & Saintilan, 2016). The overlap between Chinese and Malaysian farmed *P. monodon* samples is likely due to similar environmental conditions and formulated and natural diets. Interestingly, the Indian farmed *P. monodon* had a highly enriched $\delta^{15}N$ value when compared to other farmed samples, suggesting the prawns either had a nitrogen-enriched diet (Preston, Smith, Kellaway, & Bunn, 1996) or were exposed to anthropogenic pollution (Hobson et al., 2002; Mazumder, Saintilan, Alderson, & Hollins, 2015), which also carries an enriched $\delta^{15}N$ signature.

Multivariate, LDA and RandomForest all support the conclusion that farmed *P. monodon* samples were significantly different from wild-caught samples when using elemental analysis. This is presumably due to the diet, as well as the water quality differences between farmed and wild-caught samples. The diet and environment of marine organisms has been found to contribute significantly to the bioaccumulation of elements (Smith & Watts, 2009). Elements such as copper, cadmium, and selenium have been shown to accumulate in *Hyalella* through their diet and environment (Borgmann, Couillard, & Grapentine, 2007). Interestingly copper, which has been shown to accumulate in the muscle of *P. monodon* (Darmono & Denton, 1990; Lee & Shiau, 2002), was determined to be the most important variable when using randomForest to distinguish the production methods (farm vs. wild).

The current study found that the elemental abundance of samples is more accurate in predicting the production methods (farm vs. wild) for *P. monodon* than stable carbon and nitrogen isotopes (Table 2). This result differs to that of Ortea and Gallardo (2015) who found that stable C and N isotope values were better than elemental data in predicting the production methods of seven prawn species. It should be noted that Ortea and Gallardo (2015) only used five different elements in their analysis, whereas the current study used the relative abundance of 31 different elements. Because LDA minimises the variability within each group (e.g., Indian farmed *P. monodon*), and maximises the variability between different groups (e.g., Chinese farmed *P. monodon* vs. Chinese wild *P. monodon*), it provides the analysis with more variables that will allow for higher predictive accuracy (Pohar, Blas, & Turk, 2004). Similarly, randomForest uses the different variables present in the dataset to choose a path to follow down the decision trees until it reaches a conclusion. Therefore, using a larger dataset allowed randomForest to achieve a higher accuracy than previous work in prawn provenance using elemental analysis.

The geographic origin of *P. monodon* samples could be separated by stable carbon and nitrogen data, as revealed by the three statistical methods used in this study. Interestingly, the SIA data had a lower accuracy than elemental abundance when predicting the geographic locations in both LDA and randomForest. This result is close to the observation of Kim, Kumar, Hwang, et al. (2015), who distinguished between eight different geographic locations of 6 different prawn species. However, the LDA analysis in the present study had a higher overall accuracy (> 90%) than the one used in the study by Kim et al. (2015) (75%). In the present study, SIA was unable to distinguish between Asian and Australian prawn samples, suggesting that further studies using more samples are needed before the tool can be used for authenticating the origin of seafood.

The relative elemental abundance of *P. monodon* distinguished between their geographic locations. There is a clear distinction between the Asian and Australian samples and the nMDS revealed a distinct difference between the two regions. In a similar study, Li et al. (2014) used 20 different elements to authenticate the geographic origins of Pacific white prawns (*Litopenaeus vannamei*). The study determined that using elemental profiling showed promise by distinguishing the geographic origins of the *L. vannamei*. However, the study concluded that further development is needed before the method could be applied to authenticating the geographic origins of imported samples. The present study addressed this recommendation by detecting a larger number of elements (31 in total), and using more powerful data analyses tools ie. LDA and randomForest. The similar behaviour of the two analyses suggest that the results are robust and that XRF scanning through Itrax is the most accurate method of distinguishing the geographic locations of *P. monodon*.

The different elemental datasets used in this study had varying results. For instance, removing all elements but the most abundant elements resulted in the lowest accuracy when discriminating between farmed and wild-caught prawns and their geographic locations. Removing the elements which contributed the least to the differences had minimal effect on the accuracy, as expected. However, using only the seven essential elements picked up through the Itrax had a similar accuracy to the full set of elements. This suggests that, if it is not possible to use Itrax, then detecting essential elements, using alternative methods such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), can be effective.

The findings of this study can add significantly to the suite of tools that can be used by regulators, wholesalers, retailers and the prawn farming industry to determine provenance and production methods to satisfy certification requirements across the market chain, and to combat food fraud. Similarly, the techniques discussed here can help to reduce biosecurity risks or respond to new disease outbreaks by tracing suspected infected prawn products to their origin.

5. Conclusion

The novel application of XRF through Itrax scanning to determine the relative elemental abundance of the prawn samples makes this study unique, and has potential as a provenance tool for other seafood products. There were no common elements which distinguished between production methods (farmed and wild-caught prawns) or geographical region. All three statistical analyses could distinguish between farmed and wild-caught samples, as well as geographical origins with high accuracy. These statistical models provided consistent results, presumably due to large number of elements that Itrax detected, while most other elemental studies focus on less. Although the present study used homogenised oven-dried samples, the Itrax scanner can scan unprocessed tissue, thus, processing the samples quickly and leaving them available for other analyses. These techniques can potentially be applied to other seafood products, and also used to profile environmental determinants of chemical markers such as fish feed, fertilisers, and water quality.

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Conflicts of interest

The authors of this paper have no conflicts of interest to declare. The authors nor the organisations they work for are invested in the seafood industry.

Ethical statement

All of the samples used in the current study were provided by the research networks, who obtained samples directly from the source through fishermen, or from the farmers associations.

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