Food Control 95 (2019) 242–248

Contents lists available at ScienceDirect

Food Control

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

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

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ARTICLE INFO

Keywords:
Seafood
Provenance
Aquaculture
Elemental profiling
Stable isotopes
Black tiger prawns

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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https://doi.org/10.1016/j.foodcont.2018.08.012
Received 9 January 2018; Received in revised form 8 August 2018; Accepted 13 August 2018
Available online 17 August 2018

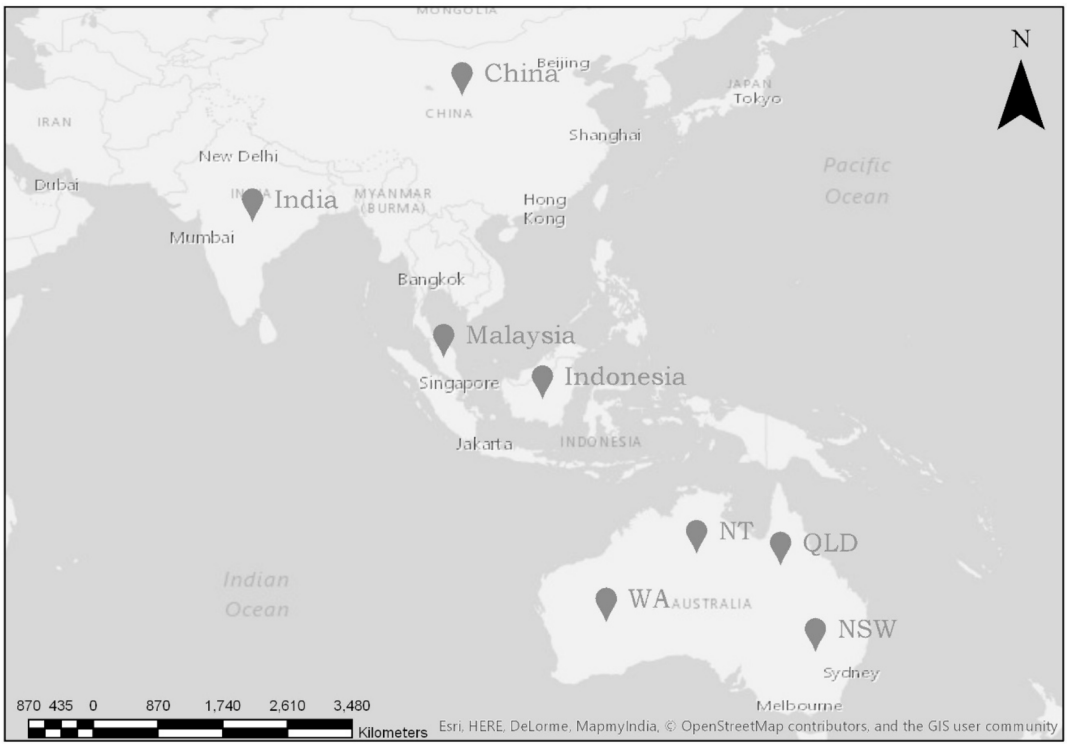

Fig. 1. Sample collection regions in Asia and Australia.

& 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