



Use of elemental profiling and isotopic signatures to differentiate Pacific white shrimp (*Litopenaeus vannamei*) from freshwater and seawater culture areas

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ABSTRACT

Shrimp samples from aquaculture ponds supplied with either freshwater (0.2–1.3 g L⁻¹ salinity) or seawater (27.6–39.0 g L⁻¹ salinity) were subjected to elemental profiling and to stable isotope analysis. Concentrations of 35 trace elements, including rare earth elements (REEs) were analyzed by ICP-MS, and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ concentrations were analyzed by IRMS in samples of peeled un-deveined (PUD) shrimp and in shrimp feed. Concentrations of 13 elements in pond water could be measured by ICP-MS. The analysis results showed that feeds offered to shrimp in freshwater and seawater did not differ in elemental concentrations ($P < 0.01$). Four elements (Li, Cr, Mn and Sr) were different ($P < 0.05$) between freshwater and seawater. The correlation between Cr in water and in shrimp was significant. Multivariate statistics including principal component analysis, stepwise discriminant analysis, canonical discriminant analysis and Kernel method demonstrated the validity of elemental profiling in distinguishing shrimp cultured in freshwater from those reared in seawater. The REEs such as Lu were more relevant to determining the provenance of shrimp than were Sr, Ba, Mn, As and $\delta^{15}\text{N}$.

1. Introduction

Shrimp is a popular seafood of high protein and low fat content. The Pacific white shrimp *Litopenaeus vannamei* has become the most common species in the global shrimp market (Boyd & McNevin, 2018). The production of *L. vannamei* increased steadily to over 3,879,786 t in 2015 (FAO, 2018a). The species is euryhaline and can be cultured over an environmental salt content range from 0.5 to 40 g L⁻¹ (Roy, Davis, Saoud, & Henry, 2007). In China, production of *L. vannamei* was 1,624,643 t in 2015 and 45% of the production was cultured in fresh or inland low salinity water (China Fisheries Yearbook, 2016).

Food products should meet the expectations of the consumers, and the taste of *L. vannamei* cultured in low-salinity water tends to be inferior to that of shrimp cultured in seawater (Liang, Wang, Wang, Chang, & Mai, 2008). In addition, microorganisms such as cyanobacteria and actinomycetes in ponds produce odorous compounds that when absorbed in the flesh render shrimp off-flavor. Low-salinity water favors the growth of cyanobacteria resulting in more frequent problems with off-flavor in shrimp cultured in low-salinity water than in seawater

(Boyd, 2003; Liang et al., 2008). There is pressing need to develop a method to differentiate shrimp cultured in low and high salinities to inform consumers of the origin of shrimp and protect their right to choose products according to personal preference.

Multi-element and stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) have been widely used in verifying geographical origin of agricultural products such as wines, cocoa beans, cabbages, rice, tea etc. (Bertoldi, Barbero, Camin, Caligiani, & Larcher, 2016; Bong et al., 2013; Chung et al., 2018; Ma et al., 2016; Martin, Watling, & Lee, 2012; Pilgrim, Watling, & Grice, 2010). These products can be traced to place of origin based on concentrations of elements such as Cu, Al, Zn and Mn etc. and rare trace elements such as Pr, Sm, Eu and Tb, etc. (Danezis et al., 2017; Li, Boyd, & Dong, 2015). Recently, these methods were used to separate fisheries and aquaculture products by geographical area of origin (Li et al., 2015; Sheikh & Xu, 2017). Elemental profiling appears to have considerable promise in verification of the geographical origin of shrimp, prawn, sea cucumber, clam, mackerel, yellow croaker, pollock, etc. (Carter, Tinggi, Yang, & Fry, 2015; Iguchi, Isshiki, Takashima, & Yamashita, 2014; Kim, Suresh, & Shin, 2015; Liu et al., 2012; Smith & Watts, 2009). Moreover,

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elemental profiling has potential to distinguish salmon, shrimp etc. from different culture methods (wild versus farmed, or conventionally versus organically farmed) as shown by Anderson, Hobbie, and Smith (2010); Ortea and Gallardo (2015); Ostermeyer, Molkentin, Lehmann, Rehbein, and Walte (2014).

Seawater has higher concentration of the major elements Na, K, Ca, Mg, S and Cl than found in freshwater, but it often has lower concentrations of the common minor elements Fe, Mn, Zn and Cu (Boyd, 2015). The rare earth elements in seawater and freshwater are different and their concentrations have been proposed as chemical markers for discrimination of whether fish reared in either saltwater or freshwater origin (Diake, 1988). In the present studies, multi-element (including rare earth elements) and stable isotope analysis were used to separate *L. vannamei* cultured in freshwater from those reared in seawater. Effects of element concentrations in feed and culture water on element profiles of shrimp also were investigated to provide theoretical support for separation shrimp cultured in freshwater and seawater.

2. Material and methods

2.1. Sample collection

The *L. vannamei* samples were collected from six ponds at each of six locations along the eastern coast in China, where most of the shrimp farms in the country are located. The geographic locations of the sampling locations are depicted in Fig. 1. Freshwater ponds were in Zhuhai, Guangdong Province (0.2 g L^{-1} salt content), Tianjin (1.1 g L^{-1} salt content), and Lianyungang, Jiangsu Province (1.3 g L^{-1} salt

content). The seawater ponds were in Huiwen, Hainan Province (27.6 g L^{-1} salinity), Haikou, Hainan Province (32.6 g L^{-1} salinity), and Dongying, Shandong Province (39.0 g L^{-1} salinity). A sample of six shrimp with individual body weights of 9.63–13.77 g were taken from each pond and were analyzed individually. Three samples of shrimp feed and pond water also were collected from each sampling location. The shrimp and shrimp feed samples were put in plastic bags and the water samples were placed in 50-mL plastic bottles and preserved with 1 mL of 1:1 nitric acid (HNO_3).

Shrimp, water and feed samples were put on ice and transported to the Key Laboratory of Mariculture, Ocean University of China, Qingdao, Shandong Province, China. Shrimp samples were carefully washed with distilled water, tails were removed and peeled but not deveined. Such shrimp are known as peeled, undeveined (PUD) in the market (FAO, 2018b). Feed and PUD shrimp samples were dried to a constant weight in a freeze drier (FD-2A, Boyikang Laboratory Instruments Co., Ltd, Beijing, China), ground with a mortar and pestle, and stored in a desiccator for analysis.

2.2. Multi-element analysis

The ground shrimp and feed samples were digested in a microwave digestion system following the method by Zhao and Zhang (2016) in which shrimp or feed samples (0.25 g) were weighed into Teflon digestion tubes. To each tube was added 6 mL of 70% HNO_3 and tubes were left to stand for 1 h. Next, 2 mL of 30% hydrogen peroxide (H_2O_2) were added to each tube, and after 30 min, the tubes were sealed and digested in a microwave digestion instrument (MARS 5 of CEM Co.,

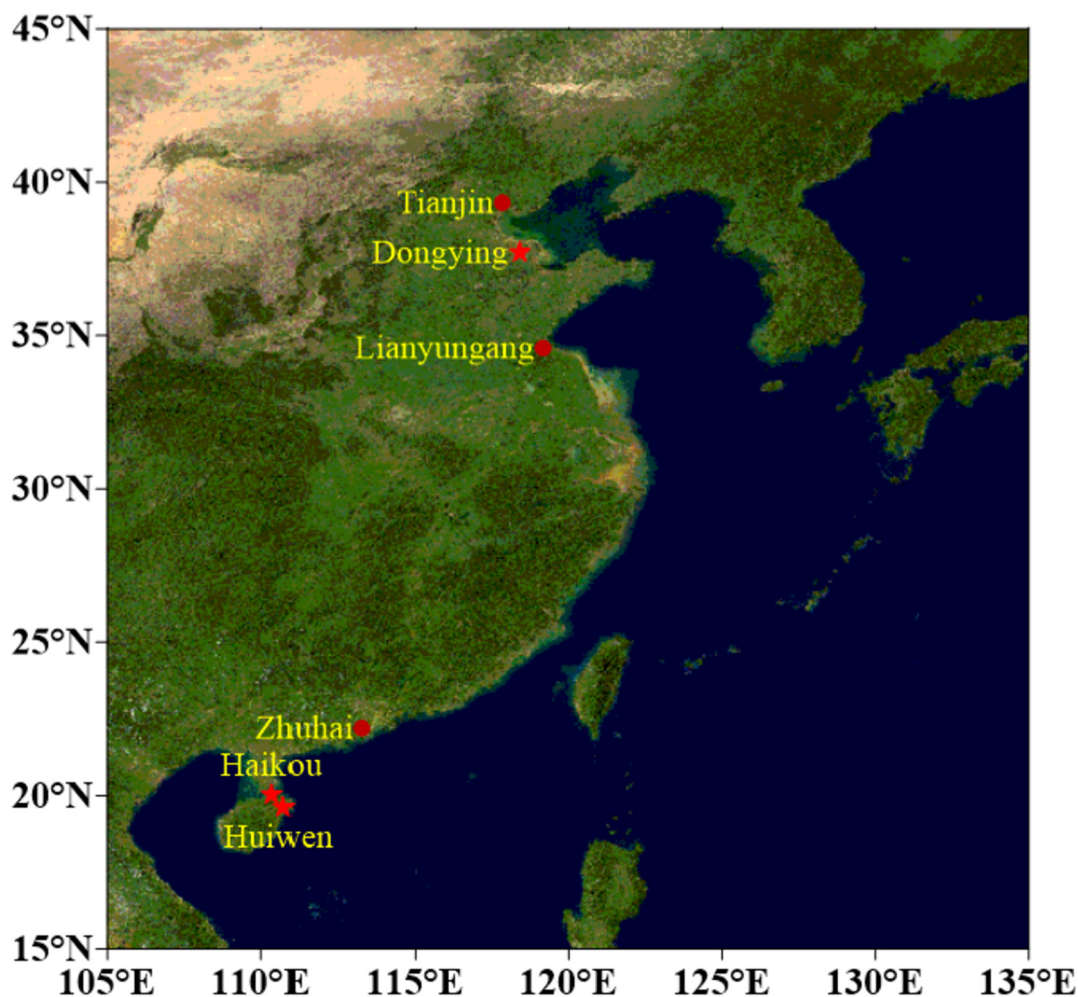


Fig. 1. Geographic locations of the sampling areas (stars = high salinity; dots = freshwater).

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