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Shrimp (sub-)national traceability and retail fraud detection using stable isotopes and multi-element profiling

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ABSTRACT

Credible seafood traceability is essential to supporting sustainability claims and enforcing import regulations designed to prevent environmental degradation and fraud. In this study, we evaluate the potential of combining stable isotope ratio and multi-elemental analyses to determine the geographic origin of whiteleg shrimp (*Litopenaeus vannamei*) meat from Ecuador (n = 191), Honduras (n = 118), and Thailand (n = 66). Reference samples of shrimp meat, telson (n = 32), pond water (n = 32), and feed (n = 32) were analyzed using isotope ratio mass spectrometry (IRMS) (δ^2H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S) and inductively coupled plasma mass spectrometry (ICP-MS) for elemental profiling. We assessed the extent to which chemically processed samples, to mimic commercial conditions, and retail samples retained their multivariate and elemental profiles comparable to reference samples. Random forest models demonstrated high accuracy for country-level classification of reference shrimp (out-of-bag error = 0.47%) and retained strong predictive power at subnational catchment levels for Ecuador and Honduras (OOB = 3.08–5.32%). Importantly, treated shrimp retained chemical fingerprints comparable to their reference shrimp meat counterparts, achieving a 100% successful assignment to subnational areas. However, retail products exhibited low assignment accuracy (16%), suggesting either post-processing alteration or false/fraudulent labeling of origin. Spearman tests among shrimp meat, telson, feed, and water revealed strong isotopic and elemental correlations. The telson samples were correctly classified to their country of origin when tested against reference models built from shrimp meat data, demonstrating that telson shell chemistry reliably mirrors the geographic signature of the edible tissue.

1. Introduction

The seafood industry faces critical challenges in ensuring sustainable practices and maintaining product authenticity throughout complex supply chains (Gephart et al., 2025). Despite the introduction of import regulations such as the US Seafood Import Monitoring Program and the European Union catch documentation requirements, and other initiatives (e.g. certification schemes, voluntary standards), achieving full supply chain traceability remains a challenge for both farmed and wild-caught products (Gephart et al., 2025; Mosnier, 2022). Shrimp are a globally important seafood commodity, representing a significant portion of international aquaculture and seafood trade (FAO, 2020a). However, shrimp farming is also a leading driver of mangrove loss in tropical regions, particularly in Southeast Asia and Central America, where extensive farming systems have replaced vast tracts of these irreplaceable habitats (Richards & Friess, 2016). This is a major

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environmental problem as mangrove forests are for coastal protection, biodiversity, and carbon sequestration (Alongi, 2002). There is therefore significant stakeholder interest in traceability systems that can credibly differentiate between high-risk source areas and those where forest protections are respected - including reflecting risk variability at a sub-national level (Boyd et al., 2021).

Complex and opaque supply chains facilitate seafood fraud, including origin or provenance mislabeling fraud (Lawrence et al., 2022). In order to incentivize best practice in producer areas, it is necessary to establish regulatory and/or voluntary market recognition for products derived from sustainable aquaculture. This in turn depends on reliable traceability systems, capable of authenticating the origin of shrimp products. Scientific methods, such as stable isotope ratio analysis (SIRA), offer promising solutions for objective origin verification in the seafood industry (Martino, Trueman, et al., 2022). Isotopic signatures in shrimp tissues reflect the chemical composition of the environment where they were raised, allowing their geographical origin to be verified with high degree of precision. Stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) have been widely used to distinguish between wild-caught and farmed shrimp, as well as to differentiate farming locations with unique isotopic baselines (Fry, 2006). Elemental profiling, or multi-element analysis (MEA), has emerged as a complementary tool to SIRA for shrimp traceability and several studies have demonstrated its potential for discerning the country of origin of shrimp (Davis, Boyd, Wakefield, et al., 2021 & 2022a, Li et al., 2017 & 2019, Ortea et al., 2015; Smith & Watts, 2009). By combining chemical analyses with advanced statistical models, these approaches enhance the resolution of origin determination and provide scalable solutions for large datasets. Such innovations not only improve the accuracy of traceability systems, but also contribute to addressing broader sustainability challenges in seafood supply chains.

While elemental profiling has been examined extensively in seafood products (Davis, Boyd, & Davis, 2021; Gopi et al., 2019a; Li Li et al., 2022), there are three key unresolved issues, especially with shrimp aquaculture products. First, element concentrations are not uniformly distributed across tissues, and so the elemental profiles of shrimp from the same location can differ depending on levels of processing (Davis et al., 2022c; Li et al., 2014). In American and European markets, shrimp are sold in a range of different forms, but most commonly whole shrimp (head-on, shell-on) and tail muscles alone (head-off, shell-off), (Davis et al., 2022d). However, even in most “peeled” shrimp products, the telson and the shell on the last abdominal segment remain. Given that the shells of shrimp are more mineralized than the muscle tissue (Davis et al., 2022c; Li et al., 2014), the prospect that the telson provides a stronger elemental signal remains a possibility. Second, little is known about the relationship between the stable isotope and elemental signature of shrimp tissue, to those of the water in the shrimp ponds and the feed used. Third, many shrimp processors use chemical treatments as preservatives, such as Sodium Chloride and Sodium Metabisulfite (SMBS) (Berardi et al., 2022). Preliminary studies have shown that these chemical treatments can alter the elemental profiles of shrimp (Davis et al., 2022b), presenting a major barrier to the use of elemental profiling in retail-facing products (Davis, Boyd, Wakefield, et al., 2021) and for implementing elemental profiling as a scalable, reliable traceability tool for farm-raised shrimp.

In this study we (1) combine stable isotope ratio data with multi-element data to differentiate chemical fingerprints for whiteleg shrimp (*Litopenaeus vannamei*) meat, the most commonly cultured penaeid species globally (FAO, 2020b), between and within Ecuador, Honduras and Thailand; (2) create treated shrimp with verified origin to establish comparability to the chemical fingerprint of reference shrimp of the same origin, and to determine whether sub-national (catchment area) traceability is possible; (3) determine whether the chemical fingerprint of retail shrimp is comparable with reference shrimp and can be successfully assigned to claimed country of origin; (4) determine whether stable isotope ratios and elements are correlated between the telson, feed and water and whether telson shell samples can be correctly assigned to country based on shrimp meat reference data. We selected Ecuador and Thailand due to their prominence in shrimp export to the United States and European Union markets. Honduras was included as a neighboring country to Ecuador to test the robustness of traceability methods when comparing geographically proximate classes, where chemical signatures may be more difficult to distinguish.

2. Materials and methods

2.1. Reference sample collection of shrimp, feed and water

Shrimp reference samples were collected from aquaculture ponds across coastal areas in Honduras (between November 2024 and January 2025), Ecuador (between October 2024 and February 2025) and Thailand (between April 2024 and March 2025). No ponds were sampled more than once, so this study did not include temporal replication. To ensure geographic representation, pond selection encompassed multiple regions within the target countries. In Honduras, samples were collected from the northern and southern areas of the Gulf of Fonseca (Fig. 1). In Ecuador, collections were conducted from the northern provinces of Esmeraldas and Manabi to the southern regions of Guayas and El Oro. Samples were collected in Thailand from the Surat Thani and Chonburi provinces, along the Gulf of Thailand, and Phang Nga, situated on the Andaman Sea coast. All ponds were located in estuaries, and the average salinity in the water content was above 10 g/L.

Shrimp farmers assisted in collecting adult shrimp samples from farm ponds using cast nets. The collected samples were between 7 and 15 cm long from head to tail, weighing 10-50g. Depending on the depth of the ponds, sampling was conducted either by wading through the water or by using a small boat. Given the dimensions of the ponds, feed was occasionally used to attract shrimp for sampling purposes. The collected shrimp were held in plastic buckets containing water from the same pond until the sampling was completed. Subsequently, the specimens were transferred to plastic bags labeled with unique identifiers to maintain the integrity of the chain of custody. At the shrimp farms, samples of feed and water were also collected. Feed and water samples were collected for 17 ponds from Ecuador, 4 ponds from Honduras and 19 ponds from Thailand (the same ponds for both the feed and water). Feed samples were obtained from the original feed bags, and pertinent information, including feed type, formulation, and batch details, was recorded. Along with the water samples, accompanying data on pond water management systems, treatment protocols, pH, and salinity levels, were also documented. Feed and water samples were directly deposited into plastic vials. GPS coordinates were recorded to accurately document the precise locations of the ponds. The packaged shrimp were placed in a cooler box with ice to preserve their integrity during transportation to the treatment center. Upon arrival, the shrimp bags were stored in a freezer until the scheduled heat treatment date. All samples were meticulously stored in plastic vials and subjected to heat treatment in convection ovens at min 80 °C to comply with German veterinary import regulations and to prevent microbial spoilage during transport. Temperature reference marker stickers were applied to record the desired heat. The treatment protocol was adjusted based on sample type: shrimp samples underwent heat exposure for 2 h, whereas feed and water samples were treated for 1 h. Vials containing different types of samples were separated to avoid cross-contamination. Following the treatment, the samples were shipped to Agroisolab GmbH (Germany) and Indikator GmbH (Germany) in accordance with all relevant export and import regulations for research purposes. For every collection (reference, retail, chemical processing, see below) and subsequent chemical analysis, three to six shrimp were merged together to create a single sample (n) of the necessary weight to allow for both SIRA and MEA. As such, the sample numbers per country are: Ecuador ($n = 191$), Honduras ($n = 118$) and Thailand ($n = 66$).

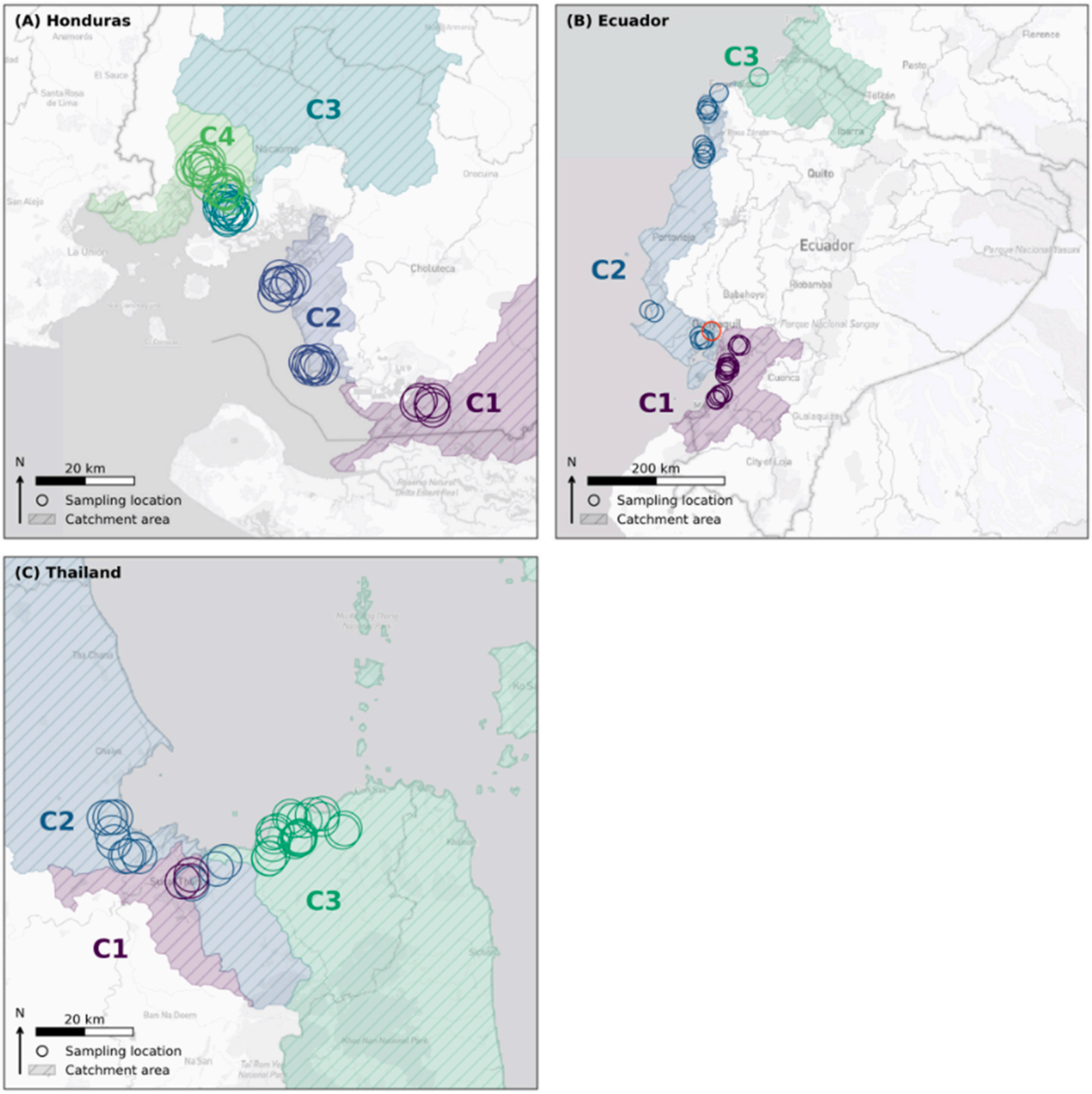


Fig. 1. *Maps of shrimp pond sampling locations in (A) Honduras, (B) Ecuador, and (C) Thailand. Note - sampling locations are fuzzified to ensure the anonymity of shrimp farmers. The catchment areas for each country are indicated ("C", see last paragraph of 2.5 Data analysis and 3.3 subnational origin assignment). The pond of the treated samples in Ecuador is indicated by the red circle (see 2.2 Retail sample and chemically processed sample collection* and *3.2 Country, treated, retail and telson origin assignment).*

2.2. Retail sample and chemically processed sample collection

A goal of this study was to investigate whether processed shrimp and/or retail samples can be traced back to their origin. To understand this we created processed samples following industry methods and purchased retail samples from commercial outlets in Germany, the United Kingdom, and the United States (Table S1). The collected retail samples were between 4 and 10 cm long from head to tail, weighing 10-50g and the samples collected to be processed were 7-15 cm long from head to tail, weighing 10-50g.

The processed samples were from known subnational locations in Ecuador (collected in July 2025), and put through a chemical process to mimic the process shrimp normally undergo before export to Western markets. Hereafter, these samples are referred to as "treated samples". A controlled collection of 80 shrimp was carried out across two aquaculture ponds (40 shrimp per pond) in Ecuador. These collected specimens were subject to standard post-harvest chemical treatments commonly employed in the industry, including exposure to SMBS, sodium tripolyphosphate (STPP), and chlorine. All chemicals used for the treatments were from the brand XRD Chemical. Per pond, samples were divided into batches of ten shrimp to undergo the three chemical processes separately (Table 1). One untreated control group served as a baseline for verification purposes. For each group of ten, two subsamples of five shrimp were created for the analysis, as such, each control group and each process had two measurements per pond.

For the SMBS treatment, 80 g of SMBS were dissolved in 100 mL of water, and the solution was adjusted to a pH of approximately 4.1–4.2. This concentrate was then diluted with 1.536 L of water (pre-cooled with 384 g of ice) to maintain the final solution temperature between 5 and 7 °C. Shrimp specimens were immersed in the SMBS solution for 10–15 min. For the sodium tripolyphosphate (STPP) treatment, 50 g of STPP were dissolved in 1 L of pre-cooled water, maintained at 0–4 °C.

Table 1
Main characteristics of the chemical treatments.

Treatment	Temperature	Concentration	Duration
Sodium metabisulfite (SMBS)	5-7 °C	38 g/kg	10-15 min
Sodium tripolyphosphate (STPP)	0-4 °C	50 g/kg	5-10 min
Chlorine	20-25 °C	20-50 mg/kg	1-2 min

Shrimp specimens were immersed in the STPP solution for 5–10 min. Finally, for the chlorine treatment, water at ambient temperature (20–25 °C) was mixed with chlorine to achieve a final concentration of 20-50 mg/kg. Shrimp specimens were immersed in the chlorine solution for 1–2 min. After the chemical treatments, SMBS and STPP samples were drained, while the chlorinated samples were rinsed with water. All samples were then stored frozen until the same heating protocol was followed as in 2.1 to ensure compliance with import sanitary regulations. For the retail samples, product labels declared countries of origin as Honduras, Ecuador, or Thailand. 61% of the retail samples were certified (19/31) by ASC, BAP, Naturland Organic, or EU Organic. All retail samples were subjected to an identical thermal processing protocol to the reference samples to ensure compliance with sanitary regulations for international shipping.

2.3. Stable isotope ratio analysis

2.3.1. Sample preparation

The shrimp meat was mechanically removed from the telson. Depending on the condition of the shrimp samples, the meat was freeze-dried to extract the tissue water. The freeze-drying, with separation and collection of tissue water, was performed using a Kryosis model (AIL Technologies, Jülich, Germany). The temperature was set to 60 °C to minimize Maillard reaction and the water was trapped with liquid nitrogen. With a vacuum of 10 mbar and heating up to 60 °C, the tissue water can be separated from up to 24 samples in one run and is completed within 4 to 6 h. This freeze-drying system guarantees the almost complete separation of water without any isotope fractionation effect. In the case of samples where separation of the tissue water was no longer feasible, the samples were simply dried in a drying cabinet at 60 °C. The telsons were dried in a drying cabinet at 60 °C. The dried samples of shrimp, feed, and telsons were then finely pulverized using a ceramic mortar, to avoid contamination, which can occur when using a ball mill with metal sleeves.

2.3.2. Stable isotope ratio analysis

2.3.2.1. Shrimp tissue water and pond water. For $\delta^{18}O$ in shrimp tissue water and pond water, a Multiflow (Elementar, Langenselbold, Germany) equilibration system and a continuous flow Isoprime (Elementar, Langenselbold, Germany) mass spectrometer were used. The liquid samples (sample volume = 200 μL) were covered with a CO_2/He mixture (reference gas with a known isotope ratio of $^{18}O/^{16}O$) and equilibrated for 5 h (30 °C, GC at 90 °C). During this equilibration period, oxygen is exchanged between the reference gas and the sample liquid. The gas is then measured individually from the sample vessels. The isotope ratio of the liquid sample is calculated from the change in the $^{18}O/^{16}O$ isotope ratio of the reference gas before and after equilibration (Epstein et al., 1953). For $\delta^{2}H$ in shrimp tissue water and pond water, a liquid autosampler hta3000A (hta, Brescia, Italy) on EA-3000 elemental analyzer (Eurovector, Pavia, Italy) as the pyrolysis unit, and a continuous flow Isoprime (Elementar, Langenselbold, Germany) mass spectrometer with $^{2}H/^{1}H$ modification were used. A chromium technique is used to determine the $^{2}H/^{1}H$ ratio (Kelly et al., 2001). To extract hydrogen from water (sample volume = 1 μL), this technique exploits the fact that chromium reduces water at 1050 °C. Any oxygen and most other elements in the water phase, such as nitrogen, sulfur, and halogens, are bound and thus removed from the carrier gas. Due to the low absorption coefficient of chromium, hydrogen diffuses unhindered through the chromium. For both $\delta^{18}O$ and $\delta^{2}H$ in shrimp tissue water/pond water, the following laboratory standards are used: 3 water samples (low, medium and high) calibrated to VSMOW2, USGS-47, GRESP and SLAP2. The measurements were carried out in duplicate at least.

2.3.2.2. Organic material. For $\delta^{18}O$ and $\delta^{2}H$ in the organic material, a zero-blank auto sampler ZB-REV2.0 (AIL Technologies, Jülich, Germany), a high temperature furnace (1550 °C)-SiC-Tube (Hekatech, Wegberg, Germany), a continuous flow Isoprime (Elementar, Langenselbold, Germany) mass spectrometer for $\delta^{2}H$, and a continuous flow HORIZON (Nu-Instruments Wrexham, UK) for $\delta^{18}O$ were used. The high temperature furnace operates with a SiC-tube (Agroisolab GmbH, Jülich, Germany, patented) to pyrolyze the samples at a temperature of 1550 °C. The SiC-tube was filled with glassy carbon and coal powder. The zero-blank autosampler was used with a heated drying chamber (revolver mode) to avoid any remaining contamination of water. The drying chamber was held at 60 °C with a steady helium flow (20 ml/min) to avoid Maillard reaction. The samples were weighed in tin cartridges (4*6 mm) and the $\delta^{18}O$ and $\delta\,^{2}H$ isotope values were determined with triplicate measurements where possible. After weighing, all samples were equilibrated for 24 h in a humidity chamber at a defined humidity (e.g. 10 %) with a known laboratory water (−7,8‰). The samples were then stored in a vacuum chamber (<100 mbar) for at least 24 h. The measurement run was continuously controlled with the laboratory standard (Dihydroxyanthraquinone) in a sequence of six laboratory standards with different weights. Two secondary standards (Benzoin and Leucine) with a difference of around 300‰ in the $\delta^{2}H$ values were also used to control the scaling. The Leucine laboratory standard was used to verify any influence of nitrogen on the $\delta^{18}O$ value. Finally, a routine sample was included in the run, to verify the consistency of the values. The measurement of the $\delta^{18}O$ and $\delta^{2}H$ was performed in a tandem setup. The dihydroxyanthraquinone laboratory standard was calibrated against international standards IAEA-601, IAEA-602, and USGS-54 for $\delta^{18}O$; and IAEA-CH7, USGS77, and USGS62 for $\delta^{2}H$.

For $\delta^{13}C$ and $\delta^{15}N$ in the organic material, a zero-blank auto sampler ZB-REV2.0 (AIL Technologies, Jülich, Germany), a EA-3000 (Eurovector, Pavia, Italy) elemental analyzer, and a continuous flow HORIZON (Nu-Instruments Wrexham, UK) were used. The samples were weighed in tin capsules (4*6 mm) and the stable isotopic values were determined in duplicate measurements. A combustion temperature of 1020 °C was set and a two-tube mode (oxidization tube filled with WO_3; Al_2O_3 on Co_2/reduction with Cu) was used. The reduction temperature was set to 640 °C. The gasses (N_2 and CO_2) were separated in a packed column (60 °C). A dilution mode was set in HORIZON to measure nitrogen and carbon dioxide in one run. Leucine was used as the laboratory standard and was controlled in the run with a routine sample. The international standards are IAEA-CH7, USGS-54, USGS-61, and USGS-62 for $\delta^{13}C$; and IAEA-N1, IAEA-N2, and USGS 61 for $\delta^{15}N$.

For $\delta^{34}S$ in the organic shrimp material, a zero-blank auto sampler ZB-REV2.0 (AIL Technologies, Jülich, Germany), a EA-3000 (Eurovector, Pavia, Italy) elemental analyzer, and a continuous flow Isoprime (Elementar, Langenselbold, Germany) were used. The combustion and reduction of SO_x was done in one tube filled with WO_3 and Cu. The water trap with magnesium perchlorate was installed directly at the outlet of the tube. The samples were weighed in tin capsules (4*6 mm) and the $\delta^{34}S$ isotope value was determined in duplicate measurements where possible. To check that the measuring system was working correctly, each measurement run was started with the measurement of 12 laboratory standards. During the measurement run, a set of 4 laboratory standards (different weighing) was measured for each of the 12 samples. To further improve combustion (1010 °C) through additional oxygen input, approximately 1 mg of extra purified vanadium pentoxide was added to the samples. Any new batch of vanadium pentoxide was checked on sulfur before use. Where possible, at least two routine samples were included to verify the consistency. The laboratory standard is cysteine trituration and the international standards are IAEA-S1, IAEA-S2, and IAEA-S3.

The internal acceptance limits for combined single-measurement uncertainty (2σ) are: $\delta^{13}C$ 0.4‰, $\delta^{15}N$ 0.4‰, $\delta^{34}S$ 0.6‰, $\delta^{18}O$ (organic) 0.8‰, $\delta^{2}H$ (organic) 4.0‰, $\delta^{18}O$ (tissue water) 0.6‰, and $\delta^{2}H$ (tissue water) 3.0‰. Measurements exceeding these limits were repeated. The total combined single-measurement uncertainty is calculated by combining reproducibility (estimated from repeated measurements of the working standard and the sample) with systematic error, which is defined by the observed bias, the stated uncertainties of the international standards, and the uncertainty introduced during standardization.

2.4. Multi element analysis

MEA was conducted by Indikator GmbH (Wuppertal, Germany) via ICP-MS using a PerkinElmer NexION 2000 instrument (Shelton, CT, USA). The ICP-MS was equipped with a Meinhard nebulizer, a cyclonic spray chamber, platinum sampler and skimmer cones, and a PerkinElmer S23 autosampler (Shelton, CT, USA). The instrument was operated at an RF power of 1200–1500 W. The plasma gas (argon) flow rate was set at 12 L/min, with an auxiliary (coolant) gas flow of 1.2 L/min, and a nebulizer (carrier) gas flow of 0.9–1.1 L/min. Approximately 400 mg of a freeze-dried, pulverized muscle sample was weighed into a microwave digestion vessel (Anton Paar, Graz, Austria). The sample was digested with 5 mL of concentrated nitric acid (65%, suprapur grade) and 100 μL of concentrated hydrochloric acid (35%, Suprapur - Carl Roth, Karlsruhe, Germany) using a closed-vessel microwave digestion system (Anton Paar, Graz, Austria). The digestion was carried out according to an optimized temperature ramp, with the goal of obtaining a fully dissolved, clear solution. After cooling, 15 mL of a diluted hydrogen peroxide solution (prepared by mixing 50 mL of 30% H_2O_2 (Suprapur - Carl Roth, Karlsruhe, Germany) with 950 mL of ultrapure water) was added to the digested samples. For the ICP-MS analysis, an external calibration was performed using a series of aqueous standard solutions. Rhodium (Rh) and Rhenium (Re) were used as internal standards to correct for matrix effects and instrument drift. The accuracy of the calibration and analytical method was verified by the analysis of a certified reference materials: TMDA-62.3 (water trace element reference from Environment and Climate Change Canada, Lot 0622) and an independent multi-element standard solution (ICP-MS standard solution CRM CR-42, Carl Roth, Germany). Both quality control samples confirmed the reliability of the measurements. The method was validated to achieve low limits of quantification (LOQs) for a crab tissue matrix, determined using the blank value method according to DIN 32645. The following elements (mass-to-charge-ratio) were measured (41 total): Aluminium (Al)-27, Arsenic (As)-75, Boron (B)-10, Barium (Ba) −137, Calcium (Ca)-44, Cadmium (Cd)-111, Selenium (Ce)-140, Cobalt (Co) −59, Chromium (Cr) −52, Cesium (Cs) −133, Copper (Cu)-65, Dysprosium (Dy)-163, Erbium (Er)-166, Europium (Eu)-153, Iron (Fe)-56, Gadmium (Gd)-157, Holmium (Ho)-165, Potassium (K)-39, Lanthanum (La)-139, Lithium (Li)-7, Lutetium (Lu)-175, Magnesium (Mg)-26, Manganese (Mn)-55, Molybdenum (Mo)-95, Sodium (Na)-23, Nickel (Ni)-60, Neodymium (Nd)-143, Lead (Pb)-206 + 207+208, Praseodymium (Pr)-141, Rubidium (Rb)-85, Antimony (Sb)-121, Selenium (Se)-82, Samarium (Sm)-149, Tin (Sn)-118, Strontium (Sr)-88, Timonium (Tm)-169, Uranium (U)-238, Vanadium (V)-51, Yttrium (Y)-89, Ytterbium (Yb)-174, and Zinc (Zn)-66. The following reference materials were used: TMDA-62.3 (Environment and Climate Change Canada) and the multi-element ICP-MS standard solution CR-42 (Carl Roth, 3A8E.1, Karlsruhe, Germany). The full quality control of this analysis and the LOQs of the measured elements are given in Supplementary Materials. For feed and water samples, a similar sample preparation and ICP-MS measurement procedure was carried out. However, water samples were analyzed without microwave digestion, and for feed the sample weight was lower to achieve good digestion. The optimization ensured complete digestion and accurate measurement. The measurements were reported in ppm or ppb (mg/kg or μg/kg) and all measurements were done in accordance with DIN EN 13805 (microwave pressure digestion) and DIN EN ISO 17294-1 (ICP-MS measurement).

2.5. Data analysis

Maps were made using Cursor with the python (version 3.10.13) packages matplotlib (Hunter, 2007), geopandas (Van den Bossche et al., 2025), and cartopy (Elson et al., 2024). All data analysis was performed in RStudio (2025) with the following packages: tidyverse (Wickham et al., 2019), ggplot2 (Wickham, 2016), randomforest (Liaw & Wiener, 2002), ranger (Wright & Ziegler, 2017), corrplot (Wei & Simko, 2021), dunn.test (Dinno, 2024), patchwork (Pedersen, 2025), and caret (Kuhn, 2008). To investigate the relationship between chemical variables, we performed Spearman's rank correlation analysis (Spearman, 1904) between the stable isotope ratio measurements and multi-element measurements separately for the shrimp meat reference samples. Next, for shrimp meat reference stable isotope ratio and multi element data, boxplots were created to show the difference between countries. As we could not plot all multi elements, Kruskal-Wallis tests (KW, Kruskal & Wallis, 1952) were performed to determine which elements are most discerning between countries and we plotted the top six. Next, we applied post-hoc Dunn tests (Dunn, 2012) to perform a pairwise country comparison and we indicate the significance levels. Next, a random forest (rf) model (Breiman, 2001) (ntree = 200, mtry = 10) was made using the shrimp meat reference stable isotope ratio and multi element data, aimed at classifying between the country levels. As there are multiple samples from each pond, it was made sure that all samples from one pond are either all in the training set (80%), or all in the test set (20%). To test for overfitting, we applied cross-validation folds (5 folds) and random labelling tests. 109 samples from Ecuador were missing the tissue water stable isotope measurements as a result of decomposition due to logistical challenges in the shipping process. These rows were deleted before any rf analysis (n = 191 -> 82). This also makes the dataset more balanced on the country level. Next, a rf model (ntree = 200, mtry = 10) was created which uses all the reference data as training data, and where the test data is either the retail or processed samples. To see if shrimp could still be assigned to a country based on telson measurements, a rf model was created using all shrimp meat reference data for training, and the telson dataset was used for testing. Although the telson dataset had no tissue water measurements (and as such we could delete that column and include more of the Ecuador samples), we still opted to use the same number of samples as the previous rf model to ensure consistency. In addition, we tested whether removing the samples for which both muscle tissue and telson were analyzed from the reference set affected performance. The accuracy of the rf model is reported via the out-of-bag error (OOB). At each iteration the samples that were not used in the training set are used to validate the current tree (Liaw & Wiener, 2002). The overall OOB accuracy is reported as the estimation of the error rate, indicating the misclassification of samples. The lower this number, the better the rf model is at correctly assigning a sample to the correct group.

To investigate whether we can detect sub-national differences in the shrimp reference stable isotope and multi element signatures, we divided each country into catchment zones. We delineated national catchment zones using the HydroBASINS dataset derived from HydroSHEDS (Lehner & Grill, 2013). Drainage basins in HydroBASINS are hierarchically subdivided following the Pfafstetter coding system, where higher levels represent finer spatial detail. We used Level 7 catchments for Honduras and Thailand and Level 6 for Ecuador to maintain adequate sample sizes. Level 6 basins (~8000–20,000 km^2) capture regional sub-basins encompassing major tributaries, while Level 7 basins (~3000–8000 km^2) represent tributary-scale watersheds with coherent hydrological and ecological characteristics. For each country, we performed KW tests to identify the most discerning elements between catchment areas. In addition, rf models (ntree = 200, mtry = 10) were developed to see if we could separate catchment areas. We evaluated the variable importance within the rf model by looking at the Mean Decrease in Accuracy (MDA) metric, and checking whether this overlapped with the initial KW test results. The MDA metric is based on the permutation of the Out-of-bag (OOB) data, and is model-driven compared to the KW test. We refer to Perrier (2015) for more detail on MDA. As one of the two ponds which were sampled to create the treated samples was located in Catchment 2 in Ecuador, we used these samples to test whether the sub-national rf model could correctly place these samples in Catchment 2.

To investigate the relationship between the chemical signatures between the shrimp, feed and water, we performed Spearman correlations between each pair for each variable. In total we used 32 samples for each type for this, as we had 8 samples that did not overlap on the pond-level. We calculate the p-value and we show the top 10 correlations for each pair, except for the feed and the water pairing, as these are unrelated to one another. For the feed comparison, we removed the Honduras data as only four different types of feed were used within the country (the same company provided the feed in each location we visited). As we had multiple shrimp sample measurements per pond, and only one feed or water measurement, we averaged the shrimp sample measurements, so we only had one measurement per pond.

3. Results

3.1. Country differences in stable isotope ratio and elemental composition for shrimp meat

The correlation matrices from the shrimp meat reference stable isotope ratio and multi element measurements are shown in Supplementary Materials Figs. S1 and S2 respectively. For stable isotope ratio measurements, the following are highly correlated ($r > 0.70$): δ^{18}O tissue water and δ^{2}H tissue water, δ^{18}O organic and δ^{2}H organic and δ^{2}H tissue water and δ^{13}C. For multi elements, we refer to the plot in Fig. S2, as a lot of elements were highly correlated. In addition, we refer to Supplementary Materials Table S2 for an overview of summary statistics per country.

Fig. 2 (A, B) shows the boxplot distribution, and Dunn test significance levels, of stable isotope measurements and multi elements per country for the reference shrimp meat data. For the stable isotope ratios, all variables were significant within the KW test and all stable isotope ratios are plotted. For the multi elements, a subselection was made based on KW test on the country level and the top six elements are shown. All elements were found to be significant within the KW test. The δ^{18}O in tissue water from Ecuadorian shrimp is noticeably more negative than values from Honduras or Thailand and there is no significant difference between Honduras and Thailand. The δ^{18}O organic is however higher for Ecuador, with again no significant difference between Honduras and Thailand. Similarly, the δ^{2}H values in tissue water from the Ecuadorian shrimp are lower when compared to Honduras and Thailand, but less pronounced for the latter. There is no significant difference in δ^{2}H organic between Ecuador and Thailand. Finally, for δ^{13}C and δ^{15}N all differences are significant, while for δ^{34}S only the differences between Ecuador - Thailand and Honduras - Thailand are significant. Looking at the top six elements, Mo and Ni are substantially lower for Honduras than Ecuador and Thailand. V is the lowest for shrimp from Thailand, while Cd is the highest. It is interesting to note the lack of variability for Cd for shrimp from Honduras when compared to Ecuador and Thailand. Co and Cr are generally higher for Ecuadorian shrimp. Dunn's post hoc test showed that for each element, all country pairs differed significantly.

3.2. Country, treated, retail and telson origin assignment

Table 2 shows the results of the rf country classification. The model achieves high accuracy (OOB = 0.47%), and all samples for the test set are correctly assigned to their respective country. The OOB when random labeling and retraining the model was around 45%, showing that the model was not overfitting. Table 3 shows the results of retraining the model with the full set of shrimp reference data (OOB = 2.75%) and testing the chemically processed and retail samples. The model can correctly assign the treated samples as Ecuadorian. For true retail samples, the model classifies 16 of the 17 retail samples with claimed origin of Ecuador to either Honduras (5) or Thailand (11) and 10 out of the 11 retail samples with claimed origin of Honduras to either Honduras (1) or Thailand (10). Most misclassifications for Ecuador and Honduras happen with Thailand, not with each other. The model assigns all retail samples with a claimed origin of Thailand to Thailand. Finally, Table 3 also shows the random forest result of the telson samples. All samples are assigned to the correct country. Removing shrimp, from which both the meat and telson were measured, from the model training set made no difference in classification accuracy.

Table 2

Random Forest country classification based on stable isotope ratio and multi element measurements (rows are reference, columns are predicted). The OOB estimate of error rate is 0.47%. The confusion matrix for both training and test set (single split reference data, processing data, retail data and telson data).

Training - Country	Ecuador	Honduras	Thailand
Ecuador	70	0	0
Honduras	0	93	0
Thailand	0	1	47
Test - Country	**Ecuador**	**Honduras**	**Thailand**
Ecuador	12	0	0
Honduras	0	25	0
Thailand	0	0	18

Table 3

Random Forest country classification based on stable isotope ratio and multi element measurements for the Retail samples (rows are reference, columns are predicted). The OOB estimate of error is 0.75%.

Training - Country	Ecuador	Honduras	Thailand
Ecuador	81	1	0
Honduras	1	117	0
Thailand	0	0	66
Treated test - Country	**Ecuador**	**Honduras**	**Thailand**
Ecuador	16	0	0
Retail test - Country	**Ecuador**	**Honduras**	**Thailand**
Ecuador	1	5	11
Honduras	0	1	10
Thailand	0	0	3
Telson test - Country	**Ecuador**	**Honduras**	**Thailand**
Ecuador	21	0	0
Honduras	0	6	0
Thailand	0	0	23

3.3. Subnational origin assignment

Fig. 3(A and B and C) show the results from the KW and rf analysis (including the MDA). For Honduras (Fig. 3A), the rf model successfully classifies samples to their catchment area (OOB = 5.32%). Both from the KW analysis and the MDA from the rf model, it appears that the elements and stable isotope ratios: As, δ^{13}C, U, Zn, δ^{2}H tissue water, and δ^{18}O tissue water are important differentiators between catchment areas. For Ecuador (Fig. 3B), the rf model is also performing well (OOB = 3.08%). We must note the class imbalance here, due to removal of samples with NA measurements for tissue water, we are left with only one sample for a Catchment 3, which makes classifying to this Catchment meaningless. However, the test set predictions for the other Catchments are accurate. The chemically processed samples are still correctly assigned to the Catchment 2 area, as the pond from which they originate is in that Catchment area (Fig. 1 - red circle on the map of Ecuador). This shows that even after processing, sub-national traceability is still possible.

Similar to Honduras sub-national differences, δ^2H tissue water and δ^{18}O tissue water appear to be important both from the KW test and MDA analysis. In addition to these, elements such as Cu seem to be returned as important by both the KW test and MDA analysis. The sub-national catchment classification for Thailand has the worst performance out of the three countries (OOB = 23.08%). There is again an imbalance between classes, although the two test samples of the smallest class are still correctly allocated to that class (Catchment 1). Again, δ^2H tissue water is brought forward as an important variable, while δ^{18}O tissue water is not returned in the top ten of the MDA list. Cr, Li and Ni are also in the top ten of both the KW and the MDA analysis.

3.4. Relationship between shrimp meat, feed, telson and water stable isotope ratio and elemental composition

Fig. 4 shows the measurement Spearman correlation results (and their significance level) between the feed, shrimp meat, telson and water samples. As expected, there are strong correlations between the shrimp and telson samples, with the highest being for Cd, δ^{34}S and Cs. Between shrimp and feed, δ^{34}S and Cd are also most highly correlated but not Cs. Elements such as K and Sb are negatively correlated but only weakly. Interestingly, between shrimp and water, the stable isotope ratio measurements in the tissue water (and the water) are not the most highly correlated. The highest correlation is for B and δ^{18}O organic. Between telson and feed, the elements Cd, As, Ni and Eu are most highly correlated. For stable isotope ratios, the most significantly correlated between telson and feed are δ^{15}N and δ^{34}S. Between the telson and water, the highest, negative correlations are for δ^2H organic and Rb and the highest positive correlation is for Sr.

4. Discussion

4.1. Spatial variability of chemical measurements in shrimp production

To ensure compliance with regulations and sustainable aquaculture standards, reliable traceability systems capable of authenticating the origin of shrimp products are required. We find clear differences in stable isotope ratios and multi element concentrations in shrimp between countries, as demonstrated by the KW test, Dunn test and boxplot analysis (Fig. 2A and B), and the strength of the rf classification model for the shrimp reference samples, which had just 0.47% OOB. Most of the existing studies on shrimp traceability use MEA as their main technique, and have reached high accuracy as well, for example 91% accuracy between five countries (Davis et al., 2022a) and 97.2% accuracy

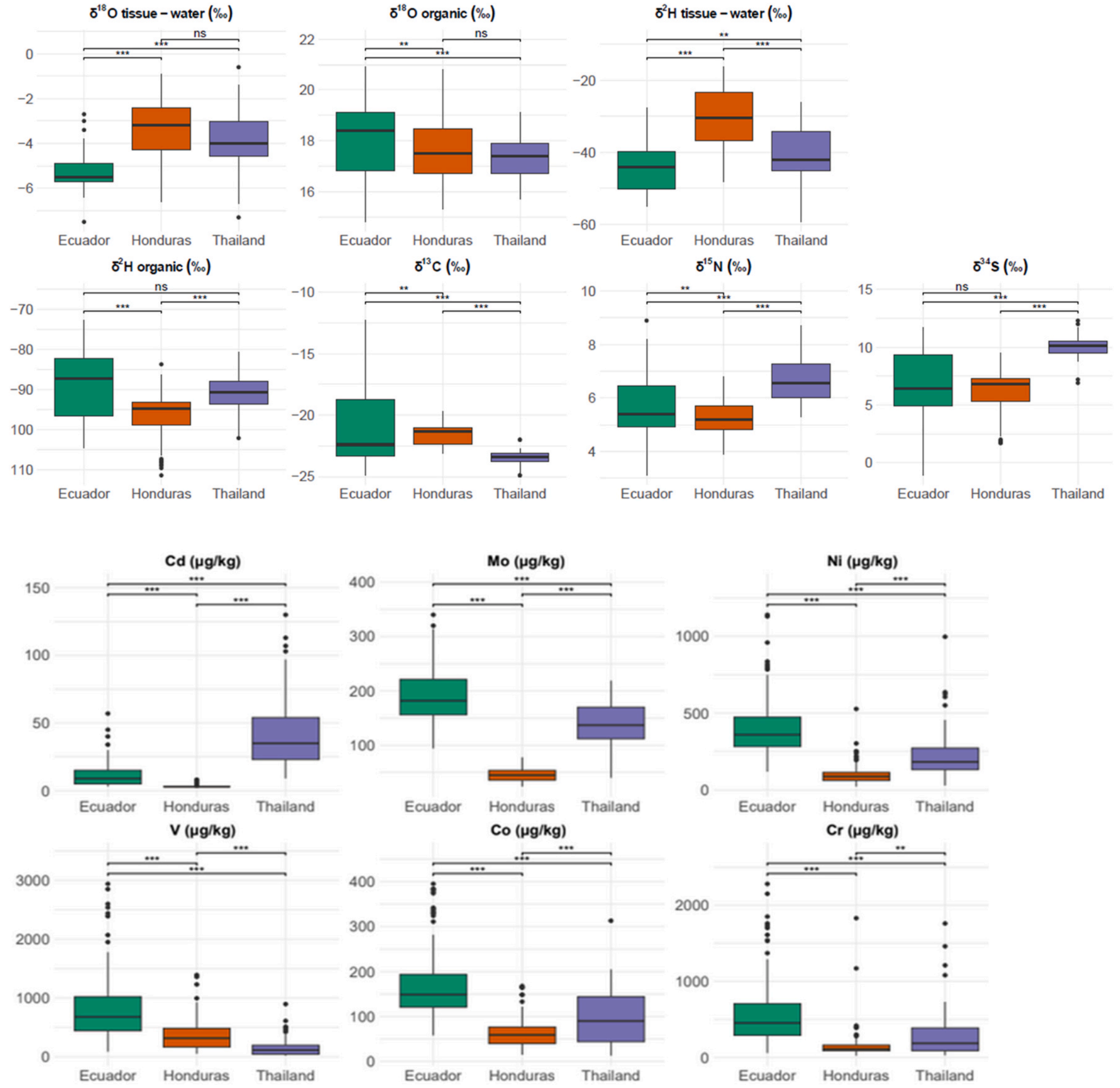
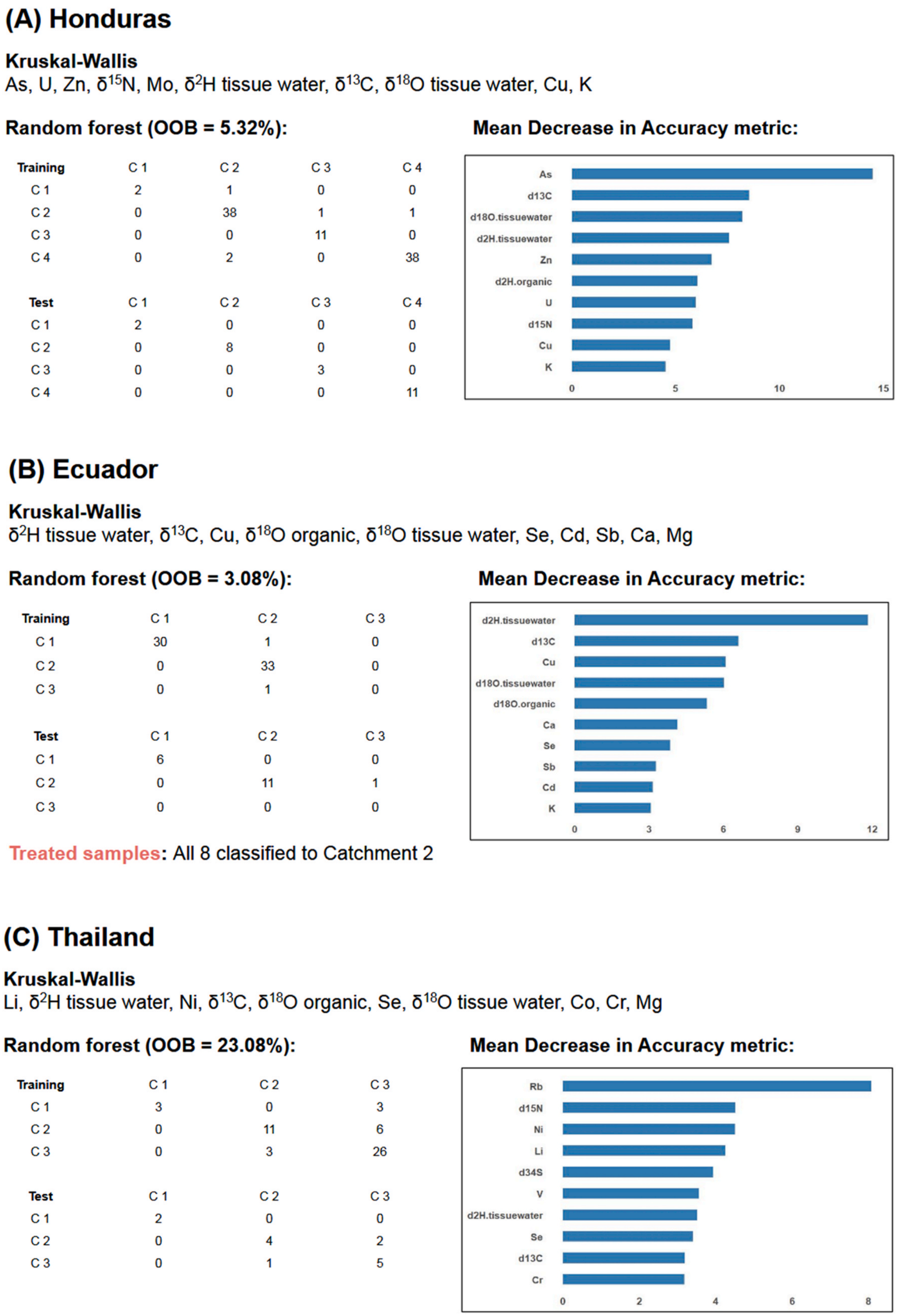


Fig. 2. (A) Boxplots for the stable isotope ratio measurements, per country, for the shrimp reference data, (B) Boxplots for the multi element measurements, per country, for the shrimp reference data. The most discerning elements between countries are shown, determined by Kruskal-Wallis.



(A) Honduras

Kruskal-Wallis

As, U, Zn, δ^{15}N, Mo, δ^{2}H tissue water, δ^{13}C, δ^{18}O tissue water, Cu, K

Random forest (OOB = 5.32%):

Training	C 1	C 2	C 3	C 4
C 1	2	1	0	0
C 2	0	38	1	1
C 3	0	0	11	0
C 4	0	2	0	38

Test	C 1	C 2	C 3	C 4
C 1	2	0	0	0
C 2	0	8	0	0
C 3	0	0	3	0
C 4	0	0	0	11

Mean Decrease in Accuracy metric:

(B) Ecuador

Kruskal-Wallis

δ^{2}H tissue water, δ^{13}C, Cu, δ^{18}O organic, δ^{18}O tissue water, Se, Cd, Sb, Ca, Mg

Random forest (OOB = 3.08%):

Training	C 1	C 2	C 3
C 1	30	1	0
C 2	0	33	0
C 3	0	1	0

Test	C 1	C 2	C 3
C 1	6	0	0
C 2	0	11	1
C 3	0	0	0

Treated samples: All 8 classified to Catchment 2

Mean Decrease in Accuracy metric:

(C) Thailand

Kruskal-Wallis

Li, δ^{2}H tissue water, Ni, δ^{13}C, δ^{18}O organic, Se, δ^{18}O tissue water, Co, Cr, Mg

Random forest (OOB = 23.08%):

Training	C 1	C 2	C 3
C 1	3	0	3
C 2	0	11	6
C 3	0	3	26

Test	C 1	C 2	C 3
C 1	2	0	0
C 2	0	4	2
C 3	0	1	5

Mean Decrease in Accuracy metric:

Fig. 3. The results of the Kurskal-Wallis and random forest + MDA analysis for (A) Honduras, (B) Ecuador and (C) Thailand. "C" indicates catchment area (see Fig. 1). For Ecuador (middle), one of two ponds from which the treated samples originated is also shown Fig. 1 (red circle). A separate random forest model was built to classify these samples to catchment area. Note - sampling locations are fuzzified to ensure the anonymity of shrimp farmers.

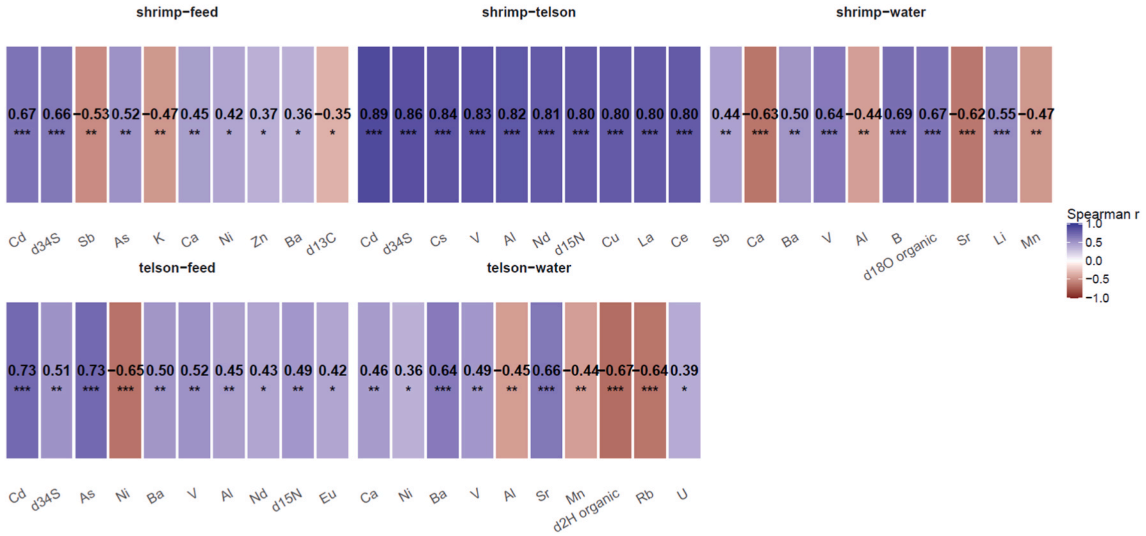


Fig. 4. For each pair, a bar plot indicating the Spearman correlation and the significant level (*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, ns = $p \geq 0.05$) for the top 10 correlations of stable isotope ratios and multi elements.

between three countries (Li et al., 2017). The study by Ortea and Gallardo (2015) used SIRA and MEA to determine the origin (Argentina, North Atlantic, Mozambique, Nigeria, Senegal and Western Central Pacific area) of commercially relevant shrimps, with 74% accuracy for SIRA, 93.5% for MEA and 100% for the combination. Other seafood or fishery traceability studies have used either SIRA or MEA, or a combination of both: black tiger prawn from several Asia-Pacific Locations with 100% for MEA, 95% for SIRA and 100% for the combination (Gopi et al., 2019b), Asian seabass with 84% accuracy for SIRA, 72% for MEA and 81% for the combination (to note that the combined model was the only model with no wrong prediction) (Gopi et al., 2019c), sea cucumber within China with 95% accuracy for MEA (Sun et al., 2022) and octopus between southeast Asia and southern Australia with 95% accuracy for the combination of MEA and SIRA (Martino, Mazumder, et al., 2022) (for a full review on this we refer to Han et al., 2022; Varrà et al., 2021). Overall, these findings suggest that both MEA and SIRA are strong standalone approaches, but that combining them should be even more powerful, since they capture different, complementary, environmental signals, for example, MEA reflecting regional elemental/geochemical conditions and SIRA reflecting isotopic signatures shaped by climate, diet, and local biogeochemical processes.

Similar to the findings of Davis et al. (2022a), Co and V were found to be important differentiators for shrimp provenance. From our subnational analysis we note that Ca (Honduras, KW and rf), Cu (Honduras and Ecuador, KW and rf), Co (Thailand, KW), Li (Thailand, rf) and As (Honduras, KW and rf) also play an important role, and these elements have been flagged by previous studies (Davis et al., 2021a, 2022a). The following will mainly impact the elemental concentration in the shrimp: the feed type (Li et al., 2016) as well as the elemental content in the water and pond sediment/soil. Wang et al. (2023) found that Cu was mainly determined by feed type, while other elements such as Cd, Pb, Cr, Ca and Li enter the shrimp via the water. In a fish pond in Vietnam a mass balance calculation showed that As was mainly introduced by influent water (Marcussen et al., 2012). Previous studies have also highlighted Al, B, Mg and Sr, which were also significant in the KW analysis for countries here. For stable isotope ratios, H and O for tissue water are more discriminatory than the organic ones. δ^{2}H and δ^{18}O in shrimp tissue water primarily record the isotopic composition of local pond and source waters, as a substantial fraction of hydrogen and oxygen in body tissues is derived directly from ambient water used for drinking and metabolic biosynthesis, causing tissue values to covary with regional water isoscapes shaped by rainfall, evaporation, altitude and distance to the coast (see also Chesson et al., 2011; Soto et al., 2016). This direct link to water sources will lead to their strong discriminatory power. At the sub-national scale, neighboring catchments can differ in the balance of riverine vs. groundwater inputs and in evaporative enrichment, generating distinct δ^{2}H and δ^{18}O signals that are transmitted into shrimp tissue water. For the other stable isotope ratios, and looking at the subnational rf models and MDA analysis, δ^{13}C is important within Honduras, δ^{13}C, δ^{15}N and δ^{34}S are important within Ecuador and δ^{13}C and δ^{15}N are important within Thailand. δ^{13}C in shrimp tissues is dominated by dietary carbon, and experimental and farm studies show that shrimp rapidly approach the δ^{13}C of their feed with relatively small, predictable trophic discrimination (Viozzi et al., 2021). So regional differences in feed formulations and basal production translate into consistent spatial structure in tissue δ^{13}C.

4.2. Comparable chemical fingerprints in treated shrimp (known origin)

A key issue with verifying the geographic provenance of shrimp is that they are sold at various levels of processing, some of which do not retain the shell. The treated samples allow us to check whether chemical fingerprints between reference shrimp samples (i.e. pre-chemical processing) and chemically processed shrimp samples are comparable, and thus whether we can match processed samples back to harvest country and/or sub-national region. Shrimp are sometimes subjected to salt baths as part of the post-harvest preparation for packaging and sales (Boyd & McNevin, 2015), including SMBS, sodium tri-polyphosphate, and sodium chloride. Element concentrations in shrimp post-processing have been found to be different to those in shrimp from the same farms that were not exposed to processing, but the effect was inconsistent between processing types (Davis et al., 2022b). However, we find the chemical signature of pre- and post-salt shrimp is still comparable, with all treated samples correctly assigned to Ecuador (Table 3), and their subnational catchment area (Fig. 3B).

While several studies have analyzed the ability of element profiling to discern country-level origins of shrimp products, there has been less emphasis on regional or subnational classification. Nonetheless, the limited studies that do address subnational classification demonstrate that elemental and isotopic markers can resolve substantial regional gradients. For example, shrimp products from various locations in the Southeastern United States have been successfully discerned (though only a few farms were included in the study; (Li et al., 2014); *Litopenaeus vannamei* and *Penaeus monodon* have been classified to specific Indonesian islands with high accuracy (∼88%; Davis et al., 2022d); Davis et al. (2022b) and Han et al. (2021) achieved a strong performance between shrimp from northern versus southern China (98.78%). In contrast, subnational differentiation within Thailand and Vietnam has proven challenging (Li et al., 2017), a pattern that aligns with the reduced performance of our Thailand catchment-level model (OOB = 23.08%). It is important to note here, however, that geopolitical boundaries determine much of the model performance (for example, the geographic distance between North and South China are far greater than Thailand and Vietnam, and there are fewer provinces within those countries). Geopolitical boundaries do generally not reflect factors that could contribute to elemental profiles, such as underlying geologic formations or watershed boundaries.

Studies examining elemental profiles in fish (e.g., Loewen et al., 2015; Ramsay et al., 2011) and other seafood products (Albuquerque et al., 2016; Bennion et al., 2019; Ricardo et al., 2015) often utilize otolith microchemistry to build classification models to watershed scale. Here, we found that water chemistry is somewhat correlated with the elemental concentrations in shrimp muscle tissue (Fig. 4) and that subnational models were accurate (76-97%, Fig. 3A, B and C) when shrimp were clustered based on which watersheds a farm occurred in. Though current concerns around shrimp traceability often are more concerned with national origin, this study demonstrates that it is possible for shrimp products to be discriminated at finer (subnational) geographic scales, which may be of interest to processors and within commercial supply chains that seek to verify the provenance and therefore footprint of their products.

4.3. *Comparable chemical fingerprints in retail shrimp (unverified origin claim)*

Though there has been considerable interest in elemental profiling and stable isotopes in aquaculture production areas (Davis, Boyd, & Davis, 2021; Gopi et al., 2019a), there have been relatively few studies which considered consumer products. Work has been done on using DNA barcoding to assess species identity mislabeling of actual seafood products, with examples of 47% mislabeling in sushi products (Willette et al., 2017), 60 to 94% of samples labeled as red snapper which turned out to be different species (Marko et al., 2004) and 31% fish species mislabeling in EU canteens (Christiansen et al., 2018). To apply chemical techniques in real market scenarios to detect origin fraud, robust testing of actual products is key. Davis, Boyd, Wakefield, et al. (2021) found considerably lower classification accuracy in samples from retail stores (~72%) than was found in samples from shrimp farms (Davis et al., 2022a). Moreover, when farm samples were used as the training set in classification models, the accuracy of classifying the claimed origin of the retail samples was only ~40% (Davis, Boyd, Wakefield, et al., 2021). Similarly, we struggled to accurately assign the retail samples in our study with an overall accuracy of ~16% (5 correct out of 31 samples, with only claimed origin Thailand scoring 100%, Table 3). Notably, for certified samples, which represented 61% of our total retail sample set, the accuracy of classification to country of origin was only 5% (1 out of 19). The main error was shrimp with claimed 'on product' origins of Ecuador and Honduras, which had chemical profiles closer to those of reference samples from Thailand. There are a number of reasons why the models may have struggled to classify retail samples successfully. Firstly, there will inherently be errors in predictive models, although previous research suggests that this is often low (Davis, Boyd, & Davis, 2021). Secondly, results may be compromised by alteration of the chemical fingerprint in retail samples by processing. We included treated samples in our study to control for this and the comparison of accurate classification between treated and retail samples suggests that processing alteration is not the source of the error. However, we cannot rule out additional or alternative chemical processes, potentially used informally or illegally, that may have impacted the results. Some work has been done on discriminating species or origins of processed seafood products using chemical techniques, for example for salmon and cod oil from different countries (Psomiadis & Tuthorn, 2022), to differentiate salted saithe from salted Atlantic cod (Oliveira et al., 2011) and to differentiate wild and organic trout and salmon from German markets (Molkentin et al., 2015). However, as mentioned above, little work has been done comparing traded, processed, products against reference databases of unprocessed samples.

Thirdly, most retail samples were *Litopenaeus vannamei*, while some were unspecified or indicated as *Penaeus vannamei*. There might be a species effect, although most of the *Litopenaeus vannamei* are not assigned to the claimed country of origin and the unspecified are assigned to Thailand. Finally, there is the possibility that the product origin claims are false or fraudulent labelling, which would lead to incorrect classification. The true origin of the retail shrimp might be from a country or area which is not included in our model. However, we cover most of the shrimp producing areas within our included countries. More importantly, even if a misclassified sample originates from a country not represented in our dataset, it still constitutes false or fraudulent labeling, because the claimed country of origin (which is represented in the model) is demonstrably inconsistent with the model's assignment.

The potential application of elemental profiling with telson material could improve discernment of geographical origin in retail samples as any chemical tampering might have a less pronounced effect on the telson. Shell tissue in shrimp is more mineralized than muscle tissue (Davis et al., 2022c; Li et al., 2014), so patterns in element concentrations are likely to be more pronounced in shell material than the tail muscle tissue. The shell of the telson is often retained even in "peeled" shrimp samples (authors personal observations, RPD), so it may provide a more universally available source of mineralized tissue for elemental profiling. Our study shows that the element and isotope concentrations found in telsons were highly positively correlated to the concentrations in muscle tissue, suggesting that the same general patterns are present in differing shrimp tissue compartments. Especially since highly discriminating elements such as Cd, Cs, V, Al, Nd, and Cu, and stable isotope ratios such as $\delta^{34}S$ and $\delta^{15}N$ are highly correlated. These strong correlations reflect the fact that both shrimp tissue and telson are supplied by the same elemental pools derived from waterborne and dietary sources, resulting in parallel accumulation patterns despite differences in tissue composition and mineralization. As mentioned above specifically, Cd is related to water intake and Cu is mainly related to the feed type (Wang et al., 2023). So when the shrimp are feeding, the Cu will be incorporated in both tissue and telson. Cu is not in the top 10 correlations between shrimp-feed and telson-feed; however, this does not preclude similar Cu uptake and incorporation into both shrimp tissue and telson from dietary sources. Especially since Cu is crucial for hemocyanin (oxygen-transport protein) within shrimp (Ji et al., 2024). Crucially, even if a reference model is built on measurements from the shrimp muscle tissue, the telson samples are still correctly classified to country (Table 3). However, our research found that the majority of retail samples no longer have their telson.

Altogether, this study reaffirms that there are still hurdles in the application of stable isotope ratio analysis and elemental profiling in retail samples, despite the strong track record of the discernability of shrimp from various geographies from culture pond (Davis et al., 2022a, Gopi et al., 2019a; Li et al., 2017, Smith & Watts, 2009).

5. Conclusion

This study demonstrates that the integration of stable isotope ratio and multi-element analyses provides a powerful, data-driven approach to authenticating the origin of shrimp and tracing their production sources across national and subnational scales. The high accuracy of country-level classification and the ability to resolve catchment-level differences highlight the spatial sensitivity of these chemical fingerprints. Treated shrimp retained their geographic chemical fingerprint even after standard industrial processing, confirming that successful source assignment is achievable for export-ready products. However, the poor alignment between the declared and assigned origins of retail shrimp emphasizes either unknown chemical processing within the supply chain or widespread origin mislabeling, even for certified products. Future implementation will require scalable laboratory workflows, continuously updated and geographically expanded reference libraries (including currently unsampled regions and multi-year sampling), and careful consideration of analytical cost and inter-laboratory harmonization.

CRediT authorship contribution statement

V. Deklerck: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **R.P. Davis:** Writing – original draft, Software, Methodology, Investigation, Formal analysis. **A. Rodriguez-Zunino:** Writing – original draft, Project administration, Methodology, Investigation, Data curation. **S. Khongtanakrittakorn:** Resources, Methodology, Investigation. **S. Hofem:** Writing – review & editing, Methodology, Investigation, Data curation. **J. Jungkeit:** Resources, Methodology, Investigation, Data curation. **A. Leyton:** Resources, Investigation. **M. Osorto-Nuñez:** Resources, Investigation. **C. Podmore:** Writing – review & editing, Project administration. **L. Prior:** Writing – review & editing, Project administration, Investigation. **C.C. Smith:** Writing – review & editing, Software, Methodology, Data curation. **B. Soule:** Writing – review & editing, Conceptualization. **J. Truszkowski:** Writing – review & editing, Methodology. **L. Van Arsdel:** Writing – review & editing, Project administration, Investigation. **S. Wahl:** Resources, Methodology, Investigation, Data curation. **P.J. Wood:** Writing – review & editing, Methodology, Investigation. **M. Boner:** Writing – review & editing, Resources, Methodology, Investigation. **J. Saunders:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Data curation, Conceptualization.

Data statement

To protect the collectors and the anonymity of shrimp farm owners, the data is available to referees for the review process and to externals based upon reasonable request via https://zenodo.org/records/17867450.

Code statement

The code is available to referees for the review process and to externals based upon reasonable request via https://zenodo.org/records/17867450.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2026.112118.

Data availability

Data will be made available on request.

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