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RESEARCH ARTICLE

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Potential use of stable isotope and fatty acid analyses for traceability of geographic origins of jumbo squid (*Dosidicus gigas*)

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Rationale: Squid is an important seafood resource for Asian and European countries. With the continuous development of processed squid products, an effective traceability system has become increasingly prominent. Here, we attempt to trace the fishery products of the main target species, jumbo squid (*Dosidicus gigas*), by using biochemical tracers.

Methods: Carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) and fatty acid profiles were identified in squid from three harvest locations in the eastern Pacific Ocean by isotope ratio mass spectrometry and gas chromatography/mass spectrometry, respectively. Comparative analysis was used to evaluate the geographic variations in tracers and to identify the suitable discriminatory variables among origins.

Results: Significant spatial variations were found in isotopic values and fatty acid profiles in squid muscle tissues, possibly because of different food availability and/or oceanographic conditions that each group experiences at a given location. The stepwise discriminant analysis indicated that $\delta^{15}N$, C16:1*n*7, C17:1*n*7, C18:2*n*6, C20:1 and C20:4*n*6 were effective variables at differentiating origin.

Conclusions: Combined use of stable isotope ratios and fatty acid analyses could trace geographic origins of jumbo squid. This study provides an alternative approach for improving authenticity evaluation of commercial squid products.

1 | INTRODUCTION

Consumers are increasingly aware of the positive effects of marine fishery products, which are associated with high levels of beneficial nutrients for human health.[1,2] Increased market demands have resulted in an intense pressure on species of commercial importance and an increasing requirement for food quality (i.e. the authenticity evaluation and origin of species).[1,3] Squid is an important seafood resource for Asian and European countries.[4,5] Jumbo squid (*Dosidicus gigas*), one of the main target species in the international market, is the most abundant

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ommastrephid squid in the eastern Pacific Ocean.[6] Throughout its large distribution range, several harvest regions are located off the coasts of western South and Central America and the Gulf of California. The commercial annual catch exceeded 1 million tons in 2014, which is almost 24% of the total fishery products of cephalopods.[7] Frozen *D. gigas* are sold for direct consumption by consumers, and a burgeoning literature documents other processed food products developed from jumbo squid, such as collagen, gel-based products, and mince.[8-10] With the continuous development of jumbo squid products, methods aimed to improve food quality and information, such as regional identity, are needed. Moreover, the market value of raw squid differs between fishing regions and it is significantly influenced by catches.[11] In particular, diversification in the abundance of jumbo squid as a result of climate change has been observed.[12]

Several methods have been applied for differentiating the origin of *D. gigas*. For example, morphometric analysis is a conventional method for squid identification.[13,14] However, this technique may not be effective for processed squid products because morphological characteristics have been removed. DNA analysis is also widely used to classify the origin of seafood,[15] although low genetic diversity has been reported among population units within *D. gigas*.[16] Biochemical tracers, such as fatty acid (FA) profiles and stable isotope ratios, have proven to be powerful indicators to identify the geographic origin of food products.[17-21] FAs are an important energy source for the growth and survival of marine organisms, and regional differences in FA profiles have been reported at the intraspecific level.[22,23] These differences are generally associated with the divergent environmental conditions (e.g. food availability) between origins, since the FAs depend mainly on the food sources.[24] In addition, the stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values, respectively) are considered as good trophic markers to understand geographic variations of *D. gigas*.[25,26] This technique is based on the fact that $\delta^{13}C$ values change little during trophic transfers, allowing identification of foraging location, while $\delta^{15}N$ values can reveal the trophic relationships, showing stepwise enrichment between trophic levels.[27] Differences in oceanographic and biogeochemical processes can cause spatial variability in the $\delta^{13}C$ and $\delta^{15}N$ signatures of squid tissues.[25,26] Therefore, the combination of fatty acid and stable isotope analyses has been an accepted method for origin traceability and authenticity of seafood products, such as distinguishing wild and farmed marine fishes and tracing the geographic origins of seafood products.[17,20,21]

The aim of this study was to examine the combined use of fatty acid and stable isotope analyses to determine the geographic origins of *D. gigas*. To accomplish this objective, fishery products of *D. gigas* from three harvest locations in the eastern Pacific Ocean were studied. The FA profiles and the $\delta^{13}C$ and $\delta^{15}N$ values of squid muscle tissues were compared among harvest locations. This study corroborates the use of biochemical tracers to identify the geographic origins of *D. gigas* and provides an alternative method for traceability of commercial squid food products.

2 | EXPERIMENTAL

2.1 | Sampling and preparation

Fieldwork was conducted on various commercial jigging cruises operating in the offshore waters of the central eastern Pacific (CEP) and off the coasts of western South America (PER, off the Peruvian exclusive economic zone (EEZ), and CHI, off the Chilean EEZ) (Figure 1). All squid were collected fresh and quickly frozen on board, then transported to the laboratory. To remove the potential influence of season on study results, the PER and CHI samples were restricted to the spring months. The CEP squid samples were randomly selected from a larger sample size because the ambient conditions in this area are similar year-round. A total of 44 individuals (CEP, n = 16; PER, n = 13; and CHI, n = 15) were used in the subsequent analyses (Figure 1).

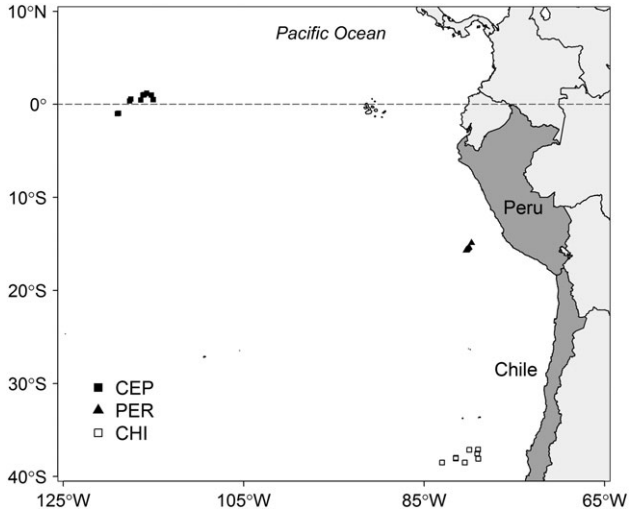

FIGURE 1 Sampling stations of *Dosidicus gigas* in the eastern Pacific Ocean. PER: off the Peruvian exclusive economic zone (EEZ); CHI: off the Chilean EEZ; CEP: offshore waters of the central eastern Pacific

Squid were measured immediately after defrosting. Approximately 2×2 cm muscle tissues were taken from the same area near funnel locking-cartilage and washed using distilled water. Each muscle piece was freeze-dried at −55°C for 24 h using a Christ Alpha 1-4 (Martin Christ, Harz, Germany), and homogenized into a fine powder using a Mixer mill MM440 (Retsch, Haan, Germany).

2.2 | Fatty acid analysis

Lipid extraction was performed according to the modified Folch method.[28] Around 200 mg powdered sample was blended in a 15-mL mixture of $CHCl_3$/MeOH (2:1, v/v) for ≥20 h. The extract was washed with 0.9% (w/v) NaCl solution and then left to stand to allow separation into two phases. The organic (lower) phase was collected and dried under N_2. The extracted lipid was dissolved in 4 mL 0.5 M NaOH/MeOH solution and refluxed for 30 min (water bath, 100°C). Thereafter, 4 mL BF_3/MeOH (14%, w/v) was added, followed by further refluxing for 30 min. After cooling, 4 mL of *n*-heptane was added and the mixture was shaken for 2 min, followed by the addition of 10 mL of a saturated NaCl solution. After each separation, the upper organic layer (FA methyl esters, in *n*-heptane) was transferred to a sample bottle prior to analysis. The FA concentrations were determined by gas chromatography/mass spectrometry (GC/MS) using a 7890B gas chromatograph interfaced with a 5977A single quadrupole mass spectrometer (both from Agilent Technologies, Santa Clara, CA, USA) and equipped with a HP-88 capillary column (60 m ×

0.25 mm × 0.20 μm, Agilent Technologies). The chromatographic separation was achieved under the following conditions: carrier gas, helium; injection volume 10 μL; injector temperature, 250°C; initial temperature, 125°C; the oven temperature was increased to 145°C at a 8°C/min and held at 145°C for 26 min, then to 220°C at 2°C/min, and held for 1 min and finally raised at 1°C/min to 227°C and held for 1 min. The split ratio was 10:1. The electron ionization (EI) source was operated in scan mode from *m/z* 50 to 500 at a source temperature of 230°C. The ionization energy and scanning frequency were 70 eV and 3 scans/s, respectively. The identification of FA methyl esters (FAMEs) was performed by comparison of retention times and full scan EI mass spectra with those of a known concentration reference standard of 37 FAMEs (GLC 37, Nu-Chek Prep, Inc., Elysian, MN, USA). The FA concentrations are reported in relation to the total concentrations of FAs (% of the total FAs), as well as the total proportion of saturated, monounsaturated and polyunsaturated FAs.

2.3 | Stable isotope analysis

Dried muscle tissue powder was directly utilized for the nitrogen stable isotope analysis. 1.5 mg of samples was added to a tin capsule prior to analysis. For carbon stable isotope analysis, lipids were extracted from the samples before analysis because lipids are depleted in ^{13}C relative to proteins and carbohydrates and the lipid concentration in muscle may vary between individuals.[18] To accomplish this, we collected the solid residues from which lipids had been removed for FA analysis. The $\delta^{13}C$ samples were then dried in an oven at 80°C for 24 h, and a 1.5-mg subsample of lipid-extracted powder was weighed into a tin capsule.

Stable isotope analysis was performed using an IsoPrime 100 isotope ratio mass-spectrometer (Isoprime Corporation, Cheadle Hulme, UK) and a vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The results are expressed in δ-notation in parts per thousand (‰) relative to international reference materials USGS 24 ($\delta^{13}C$ = −16.049 ‰) and USGS 26 ($\delta^{15}N$ = 53.7 ‰). After every ten samples, a laboratory standard (protein ($\delta^{13}C$ = −26.98 ‰ and $\delta^{15}N$ = 5.96 ‰)) was analyzed thrice with a blank sample. The analytical errors were ± 0.05 ‰ and ± 0.06 ‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

2.4 | Statistical analysis

To explore differences among geographic origins of samples, one-way analysis of variance (ANOVA) with post-hoc Tukey's honestly significant difference (HSD) test were performed on the FA profiles and isotopic datasets. To identify the most suitable discriminatory variables among geographic origins and the proportion of correct classification, FA and isotopic variables were used in the stepwise discriminant analysis (SDA), a method of linear modeling with a leave-one-out stepwise jackknife procedure. Only those FAs presented in greater than 1% in all three origins were selected for SDA because results can be biased due to many FAs not being identified in some origins (Table 1).

All statistical tests were carried out in SPSS version 19.0 (IBM Corp., Armonk, NY, USA) using a significance level of $p \leq 0.05$. All results are presented as mean value ± standard deviation (SD).

TABLE 1 Fatty acid profiles (mean ± standard error) of *Dosidicus gigas* collected off Peru (PER), Chile (CHI) and in offshore waters of the central eastern Pacific (CEP)

	N	CEP 16	PER 13	CHI 15
SFA	C14:0	0.66 ± 0.03^{a}	0.03 ± 0.02^{b}	*nd*
	C15:0	1.75 ± 0.11^{a}	0.71 ± 0.02^{b}	*nd*
	C16:0*	14.27 ± 0.92^{ab}	15.66 ± 0.31^{a}	13.07 ± 0.41^{b}
	C17:0	1.56 ± 0.08^{a}	1.46 ± 0.05^{a}	0.77 ± 0.03^{b}
	C18:0*	4.70 ± 0.11^{a}	5.55 ± 0.16^{b}	3.51 ± 0.19^{c}
	C20:0	1.24 ± 0.09^{a}	0.35 ± 0.02^{b}	1.01 ± 0.04^{c}
	C21:0	0.67 ± 0.05^{a}	0.24 ± 0.01^{b}	0.87 ± 0.03^{c}
	C22:0	0.10 ± 0.01^{a}	1.54 ± 0.09^{b}	0.17 ± 0.01^{a}
	C23:0	0.75 ± 0.06^{a}	0.74 ± 0.08^{a}	0.56 ± 0.11^{a}
	C24:0	0.22 ± 0.02^{a}	1.00 ± 0.10^{b}	0.74 ± 0.06^{b}
MUFA	C14:1*n*5	1.13 ± 0.11^{a}	0.32 ± 0.04^{b}	*nd*
	C15:1*n*5	1.12 ± 0.08^{a}	0.21 ± 0.02^{b}	0.42 ± 0.06^{c}
	C16:1*n*7*	1.11 ± 0.08^{a}	1.45 ± 0.08^{b}	1.24 ± 0.04^{ab}
	C17:1*n*7*	1.45 ± 0.15^{a}	1.38 ± 0.19^{a}	1.01 ± 0.04^{b}
	C18:1*n*9*	3.01 ± 0.18^{ab}	3.22 ± 0.22^{a}	2.42 ± 0.06^{b}
	C20:1*	3.46 ± 0.11^{a}	3.82 ± 0.13^{b}	3.28 ± 0.07^{a}
	C22:1*n*9	1.47 ± 0.10^{a}	0.92 ± 0.05^{b}	0.88 ± 0.03^{b}
	C24:1*n*9	0.16 ± 0.01^{a}	1.71 ± 0.10^{b}	0.75 ± 0.03^{c}
PUFA	C18:2*n*6*	3.41 ± 0.24^{a}	3.85 ± 0.23^{a}	4.57 ± 0.16^{b}
	C18:3*n*3	0.42 ± 0.03^{a}	1.04 ± 0.06^{b}	1.23 ± 0.10^{c}
	C18:3*n*6	0.77 ± 0.06^{a}	0.81 ± 0.11^{a}	0.95 ± 0.11^{b}
	C20:2	0.95 ± 0.06^{a}	1.03 ± 0.05^{a}	1.30 ± 0.04^{b}
	C20:3*n*3	1.76 ± 0.12^{a}	1.31 ± 0.06^{b}	*nd*
	C20:3*n*6	*nd*	1.22 ± 0.07^{a}	0.22 ± 0.03^{b}
	C20:4*n*6*	6.03 ± 0.18^{a}	2.45 ± 0.11^{b}	6.99 ± 0.22^{c}
	C20:5*n*3* EPA	8.81 ± 0.21^{a}	7.85 ± 0.14^{b}	8.10 ± 0.19^{b}
	C22:2*n*6	1.58 ± 0.25^{a}	0.45 ± 0.24^{b}	0.18 ± 0.12^{c}
	C22:6*n*3* DHA	37.43 ± 1.99^{a}	39.67 ± 0.92^{a}	45.74 ± 0.64^{b}
	n6	11.03 ± 0.44^{a}	7.49 ± 0.40^{b}	11.96 ± 0.40^{a}
	n3	48.42 ± 1.82^{a}	49.87 ± 0.94^{a}	55.07 ± 0.60^{b}
	n3/n6	4.30 ± 0.33^{a}	6.00 ± 0.47^{b}	4.34 ± 0.18^{a}

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; *n6*: omega-6 fatty acids; *n3*: omega-3 fatty acids. N: number of samples; *nd*: not detected;

*: fatty acid present in greater than > 1% in all sites.

Values in each row followed by different alphabetic characters were statistically different (one-way analysis of variance (ANOVA) with post-hoc Tukey's HSD test).

3 | RESULTS

3.1 | Stable isotope ratios

No difference in mantle length was observed between *D. gigas* captured from three locations in the eastern Pacific Ocean (CEP: 30.3 ± 3.6 cm, PER: 33.9 ± 9.9 cm, CHI: 35.5 ± 3.3 cm, ANOVA, $F_{2,41}$= 2.36, p = 0.11). However, the $\delta^{13}C$ and $\delta^{15}N$ values were significantly different among geographic origins (ANOVA, $\delta^{13}C$: $F_{2,41}$= 66.49, p <0.01; $\delta^{15}N$: $F_{2,41}$= 106.04, p <0.01). The squid from CEP (−17.58 ± 0.21 ‰, range −17.87 to −17.0 ‰) had more negative $\delta^{13}C$ values than individuals from PER (−16.15 ± 0.45 ‰, range −17.07 to −15.45 ‰) and CHI (−16.89 ± 0.33 ‰, range −17.44 to −16.23 ‰). In addition, higher $\delta^{15}N$ values (13.48 ± 2.05 ‰, range 12.25 to 15.80 ‰ and 15.49 ± 2.05 ‰, range 11.67 to 18.40 ‰, respectively) were found in squid captured from PER and CHI than from CEP (8.48 ± 0.74 ‰, range 7.25 to 9.66 ‰). However, a slight overlap was observed in convex hulls between PER and CHI (Figure 2).

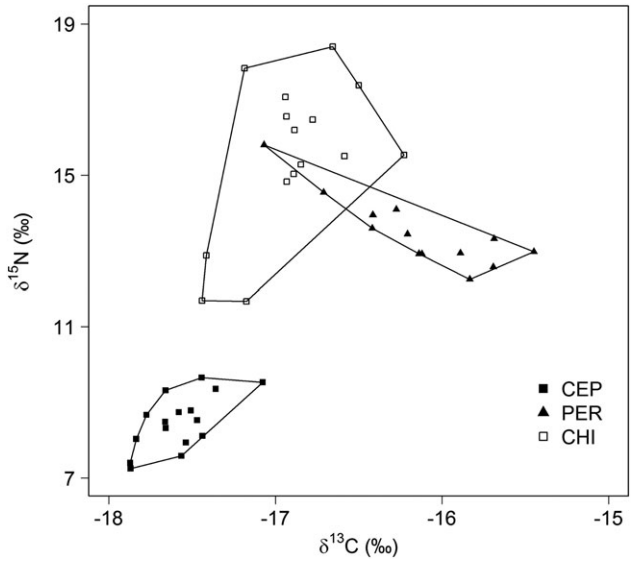

FIGURE 2 Stable carbon and nitrogen isotope ratios of *Dosidicus gigas* from PER (off the Peruvian exclusive economic zone (EEZ)), CHI (off the Chilean EEZ), and offshore waters of the central eastern Pacific (CEP)

3.2 | Fatty acid profiles

The FA profiles of jumbo squid muscle from three geographic origins are shown in Table 1. A total of 28 FAs were detected, with 10 FAs present at greater than 1% in all regions (as indicated by '*' in Table 1). These ten FAs accounted for 76.88–91.97 % of all the FAs.

Ten kinds of saturated fatty acids (SFAs) were detected in the mantle muscle of *D. gigas* from CEP and PER, and eight in specimens from CHI (Table 1). The total amount of SFAs in CHI squid (20.71 ± 0.27 %) was significantly lower than in those of specimens from CEP and PER (25.93 ± 0.96 % and 27.28 ± 0.44 %, respectively, ANOVA, $F_{2,41}$ = 28.95, p <0.01). Most of the SFAs showed different amounts among areas, except for tricosylic acid (C23:0). In all origins, the most abundant SFA was palmitic acid (C16:0) which represented over 50% of all SFAs (Table 1).

Different types of monounsaturated fatty acids (MUFAs) were detected in the three regions; e.g., myristoleic acid (C14:1*n*5) was not observed in CHI squid (Table 1). The ANOVA results showed that all the MUFAs were significantly influenced by geographic origin. The total amounts of MUFAs in CEP and PER squid (12.91 ± 0.70% and 13.04 ± 0.38 %, respectively) were higher than those in CHI individuals (10.01 ± 0.23 %, ANOVA, $F_{2,41}$ = 11.12, p <0.01). Moreover, the observed proportion of MUFAs was less than the SFAs or PUFAs in each region (Table 1).

Polyunsaturated fatty acids (PUFAs) were the dominant FAs in *D. gigas* muscle tissues, accounting for 59.68–69.28 % of all FAs. Significant spatial variations were found in all PUFAs, and the highest total content of PUFAs was present in squid from CHI (Table 1). Of the ten PUFAs detected, relatively higher proportions were observed of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) (Table 1). As an important indicator to describe the FA profiles, the ratio of omega-3 and omega-6 fatty acids (*n*3/*n*6) showed significant differences (ANOVA, $F_{2,41}$ = 7.88, p <0.01). The maximum *n*3/*n*6 values occurred in PER (6.00 ± 0.47) while the values for CEP and CHI were 4.30 ± 0.33 and 4.34 ± 0.18, respectively.

3.3 | Discriminatory variables

To identify variables that can distinguish the geographic origins of *D. gigas*, the $\delta^{13}C$ values, $\delta^{15}N$ values, FAs and *n*3/*n*6 were in combination subjected to stepwise discriminant analysis (SDA). Only ten of the most abundant FAs (mean proportions greater than 1 % in all regions, as indicated by '*' in Table 1) in squid were selected for SDA. The SDA results revealed six variables that can be used to distinguish the geographic origins ($\delta^{15}N$, C16:1*n*7, C17:1*n*7, C18:2*n*6, C20:1, C20:4*n*6). Squid captured from three regions were effectively identified with a cross-validated classification rate of 100 % (Figure 3).

4 | DISCUSSION

Stable isotope and FA analyses are considered as effective methods to trace seafood products, such as fishes,[17,18] shrimp,[19] and sea cucumber.[21] With the rapid development of commercial squid products, effective approaches aimed to track processed squid are needed. In this study, the biochemical tracers (FA profiles, $\delta^{13}C$ and $\delta^{15}N$ values) were identified for the commercial targeting squid species, *D. gigas*, to infer their geographic origins in the eastern Pacific Ocean.

Our results showed that the $\delta^{13}C$ and $\delta^{15}N$ values of squid muscle tissues were different among origins. These differences are probably due to the variation in food composition used by squid in different regions, since $\delta^{13}C$ values can reveal their food source.[29] In addition, higher $\delta^{15}N$ values suggest that squid captured from PER and CHI might feed at a higher trophic level than individuals from CEP (Figure 2). The average difference in $\delta^{15}N$ values of squids between CEP and CHI is 7.01 ‰, greater than 2 trophic levels assuming that 2.75 ‰ is the trophic enrichment factor between squid and their food.[27] Another possible explanation of such variations in isotopic ratios could be the differences in oceanographic and

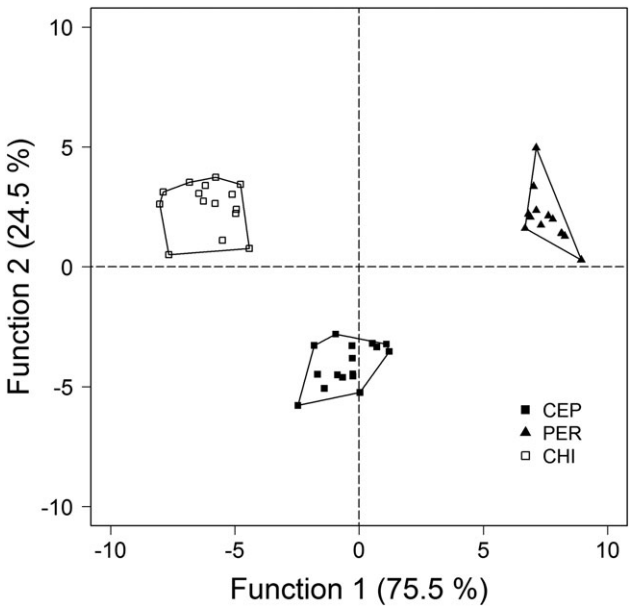

FIGURE 3 Stepwise discriminant analysis plots showing the distribution of *Dosidicus gigas* from three regions: PER (off the Peruvian exclusive economic zone (EEZ)), CHI (off the Chilean EEZ), and offshore waters of the central eastern Pacific (CEP)

biogeochemical conditions.[30,31] Compared with less productive offshore waters (i.e. CEP), higher $\delta^{13}C$ and $\delta^{15}N$ values are associated with high productivity in near-shore waters, such as the nutrient-enriched Humboldt Current and upwelling regions (i.e. PER and CHI). These spatial variabilities affect the $\delta^{13}C$ and $\delta^{15}N$ baseline values and can be propagated up in the squid's tissues through the diet.[26,32] Moreover, latitudinal gradients in $\delta^{13}C$ and $\delta^{15}N$ values have also been observed in the eastern Pacific Ocean.[25,26] For instance, differences of nearly 4.00 ‰ in $\delta^{13}C$ values and 8.00 ‰ in $\delta^{15}N$ values were detected in *D. gigas* muscle tissues along a 15° latitudinal gradient in the Humboldt Current Ecosystem.[25] Although spatial variations were found in the isotopic ratios, there is still considerable overlap between the PER and CHI convex hull areas (Figure 2), which indicates that $\delta^{13}C$ and $\delta^{15}N$ values alone cannot be used to distinguish all origins.

D. gigas varied in their FA profiles for the three geographic origins, showing the effectiveness of these profiles for discriminating the origins. Considering the environmental conditions (e.g. sea surface temperature and food availability) that each group experiences at a given location, we conclude that our results can be explained by these environmental differences. CEP is affected by a warm Equatorial Counter Current and cooler south Equatorial Current (Figure 1), while PER and CHI are influenced by the cold Humboldt Current and strong upwelling of cool subsurface waters.[33,34] In general, being a higher latitude region, CHI has a lower sea surface temperature. Upwelling of nutrient-enriched waters from the Humboldt Current supplies sufficient nutrients to regions off Peru and Chile (i.e. PER and CHI), while central equatorial Pacific waters (CEP) have lower primary productivity.[33] The differences in ambient conditions in each region may consequently result in spatial variations in FA profiles. For example, several studies have shown that sea surface temperature and food availability may influence *D. gigas* energetic requirements for growth or reproduction.[35,36] As the functional storage lipids, SFAs are synthesized to provide an adequate energy source for growth and survival of marine organisms.[22,23] Hence, geographic variations in SFAs are probably due to the different energy demands of squid inhabiting distinct oceanographic areas. Nonetheless, palmitic acid (C16:0) was identified as the basic SFA in all samples. The results are in good agreement with the observed dominance of palmitic acid in *D. gigas* mantle and arm muscle off northern Peru.[37] This trend has also been reported for other squid species.[29,38] Palmitic acid is particularly useful for growth as a preferential energy source for metabolism, and high amounts of palmitic acid favor rapid growth of *D. gigas*.[39] PUFAs were identified as the basic FAs in *D. gigas* muscle tissues. These results have been recently reported by other researchers,[29,38] and observed in *D. gigas* liver.[37] The region-specific content of PUFAs in squid tissues could correspond to their dietary sources (i.e. food availability). This is because of the inability of high trophic level predators to synthesize longer-chained PUFAs, such as DHA and EPA.[24] Indeed, differences in prey items always been found in population units within *D. gigas*. For example, the main prey of *D. gigas* off Chile is pelagic fishes,[40] whereas cephalopods are the dominant food sources in *D. gigas* which are found in the waters off Peru.[41]

In this study, stepwise discriminant analysis revealed a high rate of correct classification of *D. gigas* (Figure 3). These results indicate that stable isotopes and FAs are suitable tracers for separating the *D. gigas* into geographic groupings. These results are also in agreement with several studies in which a combination of FAs and stable isotopes was used to discriminate the origin of seafood products.[17,21] Thomas et al[17] used FA and stable isotope analyses to distinguish wild or farmed origins of Atlantic salmon (*Salmo salar*). The authors found that $\delta^{15}N$ values in choline and $\delta^{18}O$ values measured on total oil were the most suitable variables for tracing salmon to their location. Fatty acids, on the other hand, were less useful parameters. To identify the geographic origins of sea cucumbers (*Apostichopus japonicus*), Zhang et al[21] used a combination of FA composition and stable isotope ratios from the body wall of individuals. In their study, overlaps in origin were observed when $\delta^{13}C$ and $\delta^{15}N$ values were used alone, but a complete separation of origin was demonstrated when combined with FAs. This corresponds to our findings, where an overlap was observed between the PER and CHI isotopic ratios (Figure 2), and the identification of origin was improved by the addition of the FA data (Figure 3). Another interesting finding is that 18:2*n*6 and 20:4*n*6 were two of the most important variables to distinguish *D. gigas* captured from different areas, whereas Stowasser et al[29] reported that these two FAs were not affected by the diet of squid *Lolliguncula brevis*. This difference is thought to depend on different abilities of synthesis, uptake and retention of certain FAs, because *D. gigas* has much higher growth and metabolic rates than *L. brevis*.[6,42]

5 | CONCLUSIONS

Our study used comparative analyses of fatty acid composition and stable isotope ratios in *D. gigas* muscle to evaluate variability in geographic origins and to identify the most suitable discriminatory variables among them. Both FAs and isotopic ratios showed spatial variability between squid captured from three harvest locations in the eastern Pacific Ocean, and the combined use of FA composition and stable isotope ratios traced jumbo squid origins. We propose that the observed variations could be the result of differences in food availability and/or oceanographic conditions. Future work is needed to confirm the exact relationship between the *D. gigas* FA profiles and variations of the environment. Above all, these results are relevant for commercial squid food products. A combination of FA and stable isotope analyses is an alternative method for origin traceability of pelagic squid and for ensuring food quality.

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