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Discrimination of different forms and oceanic regions of purpleback flying squid (*Sthenoteuthis oualaniensis*) based on stable isotopes and fatty acid composition

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Introduction: Pelagic cephalopods play a key role in the Chinese food supply. The market value of seafood frequently fluctuates based on its geographical origin and species, making it imperative to trace the origin and distinguish forms to guarantee food quality and accurate information.

Methods: In this study, we used biochemical tracers, including stable isotope analysis and fatty acid composition profiling, to trace the origin of purpleback flying squid (*Sthenoteuthis oualaniensis*) from the Indian Ocean and South China Sea. We measured the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and fatty acid (FA) contents in samples from different forms of *S. oualaniensis* obtained from these two oceanic regions. We analyzed the feeding and nutritional differences among these populations and conducted discriminant analysis.

Results and discussion: Significant regional and form differences were observed in both isotopic values and FA profiles in the muscle tissues of *S. oualaniensis*. Forms with larger sizes exhibited higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, potentially resulting from different feeding preferences and geographical environments. The discriminant analysis revealed that isotopic composition could effectively distinguish individuals from different oceanic regions, whereas FA composition was more effective in distinguishing different forms within the same oceanic region. The combination of isotopes and FAs could accurately discriminate *S. oualaniensis* of different forms and from different oceanic regions, achieving a discrimination accuracy of 100%. This study provides a reference for tracing the origin of pelagic cephalopods with complex form structures.

KEYWORDS

Sthenoteuthis oualaniensis, stable isotopes, fatty acid, morphological discrimination, South China Sea, Indian Ocean

Abbreviations: IO, Indian Ocean; SCS, South China Sea; FA, fatty acid; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; MF, medium form; DF, dwarf form; GF, giant form; SDA, stepwise discriminant analysis.

1 Introduction

As a crucial pelagic fishery resource, the purpleback flying squid (*Sthenoteuthis oualaniensis*) has a prominent position in global fisheries and a widespread distribution, with major fishing grounds encompassing the Ryukyu Islands, the Gulf of Aden, and the northwestern Indian Ocean (IO) (Zuyev et al., 2002). Abundant resources of *S. oualaniensis* are found in the South China Sea (SCS) and IO (Zhou et al., 2008; Zhang et al., 2018) and have substantial developmental potential. The potential of this resource becomes even more evident with the current decline facing coastal fishery resources. *S. oualaniensis* is regarded as a high-quality seafood raw material because of the high-protein and low-fat characteristics of the muscle tissue. Despite challenges such as the excessive sour taste and high endogenous formaldehyde content (Fu et al., 2020), diverse applications, including processing into dried products and fish feed, provide favorable commercial markets. With the increasing harvesting of *S. oualaniensis*, the regulation of the subsequent food quality and safety has become crucial. However, the complex form structure (Staaf et al., 2010) and diverse product specifications of *S. oualaniensis* pose challenges for market supervision. The market value of seafood often varies depending on the origin and species. Consequently, origin tracing and form discrimination have emerged as crucial means to ensure food quality and information transparency.

Traditional methods for cephalopod form discrimination primarily rely on morphology and molecular biology (Lü et al., 2013; Gong et al., 2018). However, morphology-based approaches are unsuitable for processed squid products. Although molecular biology studies have revealed significant genetic differentiation among different forms of *S. oualaniensis*, uncertainty remains in the analytical results (Liu et al., 2008, 2017; Li et al., 2019). Form discrimination methods based on isotopes and fatty acids (FAs) have recently emerged as promising approaches, serving as valuable complements to cephalopod form identification (Kim et al., 2015; Ortea & Gallardo, 2015). Isotope and FA composition in organisms is strongly influenced by food sources and species accumulation processes and are therefore effective tools for form identification and habitat tracing (Spangenberg, 2016; Zhang et al., 2017).

Carbon and nitrogen stable isotopes can reflect the accumulation of feeding information in organisms, and their stable enrichment phenomena reveal the organic matter sources and food composition of consumers within the food web (Peterson and Fry, 1987). These isotopes undergo fractionation and transformation processes within organisms (Dubois et al., 2007), which can reveal the transfer of organic matter among biological tissues. The isotopic signatures of different tissues reflect feeding conditions across various time scales, making isotopic composition an ideal indicator of the living environment of an organism. Furthermore, FAs play a crucial role in marine ecosystems as essential nutrients and energy sources for marine animals. Marine animals have a limited ability to synthesize and modify FAs, particularly for highly unsaturated FAs, and they must therefore obtain these FAs from their prey and maintain the original structure of the FAs within their tissues (Parrish et al., 2000; Kharlamenko

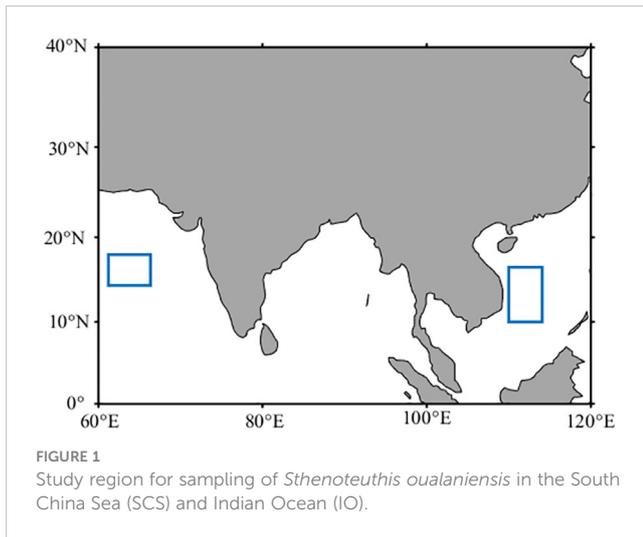
et al., 2001; Chaguri et al., 2017). Consequently, FAs have emerged as an important biomarker for tracing the flow of substances in marine ecological food webs (Raclot et al., 1998). The composition of FAs within organisms can reflect their living environments (Kasai, 2003; Hasegawa et al., 2014), and the muscles of cephalopods, as their main energy storage tissues, contain food information from a specific period. In addition, environmental factors can produce significant differences in the FA composition of muscle and hard tissues of cephalopods from different oceanic regions (Gong et al., 2018; Zhu et al., 2018).

Different forms of *S. oualaniensis* are recognized, including the medium form (MF) distributed in all oceanic regions, the dwarf form (DF) primarily found in equatorial waters, and the giant form (GF) primarily inhabiting the IO (Nesis, 1993). Previous studies have posited that GF is merely a plastic phenotype of MF, while many scholars speculate that the DF may represent an independent species distinct from the MF (Clarke, 1965; Snyder, 1998). However, molecular analyses have revealed that GF and DF both exhibit considerable genetic distances from MF, indicating a high probability of their being mutually independent species (Li et al., 2019; Xu et al., 2020; Jeena et al., 2023). This intraspecific diversity, observed in *S. oualaniensis*, is echoed in other cephalopod species as well (Fernández-Álvarez et al., 2021). These forms exhibit morphological, reproductive, and ecological variations (Jereb & Roper, 2010). Owing to the diversity of its habitat, *S. oualaniensis* from different oceanic regions displays significant differences in reproduction, growth characteristics, and other aspects (Yatsu et al., 1997; Chen et al., 2007; Chembian & Mathew, 2014; Lin et al., 2015). For species with complex form structures and significant habitat variations, isotopic and FA composition can reveal the nutritional characteristics of different forms and geographical populations, thereby enabling the tracing and tracking of such groups. The present study used isotope and FA techniques to trace the geographical origin of *S. oualaniensis* from the IO and SCS. By collecting samples from different forms in these two regions and measuring their population-level $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as well as their FA contents, this study aimed to reveal differences in feeding habits and nutritional ecology among the various populations. The successful application of isotope and FA techniques in form identification and geographical tracing will provide valuable references for tracing the origin of oceanic cephalopods with complex form structures.

2 Material and methods

2.1 Sample collection and sample pretreatment

S. oualaniensis specimens were collected on various commercial jigging cruises operating in the IO (14°33'N–18°55'N, 61°21'E–63°57'E) from November to December in 2022 and light falling-net fisheries in the SCS (9.80°N–17.25°N, 110.25°E–115.02°E) from May to June in 2022 (Figure 1). All specimens were collected fresh, immediately frozen at -20°C , and then transported to the



laboratory. A total of 79 specimens were randomly sampled. Specific population numbers and mantle lengths of four populations are detailed in Table 1.

After being defrosted in the laboratory at room temperature, *S. oualaniensis* specimens from the SCS were categorized into DF and MF groups based on the absence or presence of dorsal photophores (Zhu et al., 2016; Wang et al., 2019), whereas specimens from the IO were categorized into GF and MF groups based on the distribution range of mantle length in sexually mature individuals (Snyder, 1998; Zuyev et al., 2002). The dorsal mantle lengths were measured to the nearest 1 mm, and body weights were measured to the nearest 1 g. Muscle tissues (approximately 2 × 2 cm) were excised from the same area near the funnel locking-cartilage, washed using distilled water, and then frozen at -35°C for subsequent analyses. Each muscle piece was freeze-dried at -55°C for 48 h using a Christ Alpha 1-4 (Martin Christ, Harz, Germany), pulverized using a Mixer mill MM440 (Retsch, Haan, Germany), and then stored under dry conditions.

2.2 Stable isotope ratio analysis

Dried muscle tissue powder (1 mg) was used for analysis. After extracting lipids from samples, the carbon stable isotope was analyzed on an elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). To accomplish this, solid residues were

TABLE 1 Sample composition for the dwarf form (DF) and medium form (MF) of *Sthenoteuthis oualaniensis* in the South China Sea and giant form (GF) and MF of *S. oualaniensis* in the Indian Ocean.

Form	Range of mantle length (minimum~maximum)/mm	Mean mantle length (mean ± SD)/mm
DF CSC(N=25)	91~120	102 ± 8
MF CSC(N=24)	164~206	183 ± 12
MF IO(N=9)	200~272	231 ± 22
GF IO(N=21)	347~479	414 ± 25

collected and fat removed for FA analysis. The $\delta^{13}\text{C}$ sample was then dried in an oven at 30°C for 24 h, and 1 mg of lipid-extracted powder subsample was weighed in a tin capsule.

The $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ natural abundance is expressed in δ -notation in parts per thousand (‰) relative to international reference materials USGS 24 ($\delta^{13}\text{C} = -16.049\text{‰}$) and USGS 26 ($\delta^{15}\text{N} = 53.7\text{‰}$). After every 10 samples, a laboratory standard (protein: $\delta^{13}\text{C} = -26.98\text{‰}$ and $\delta^{15}\text{N} = 5.96\text{‰}$) was analyzed three times with a blank sample. The analytical error was $\pm 0.05\text{‰}$ and $\pm 0.06\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively.

2.3 FA analysis

Lipid extraction was performed according to the modified Folch method. Approximately 200 mg of powdered sample was blended in a 15-mL mixture of $\text{CHCl}_3/\text{MeOH}$ (2:1, v/v) for ≥ 20 h. The extract was washed with a 0.9% (w/v) NaCl solution and then left to stand for separation into two phases. The organic (lower) phase was collected and dried under N_2 . The extracted lipid was dissolved in 4 mL of 0.5 M NaOH/MeOH solution and refluxed for 30 min (water bath, 100°C). Thereafter, 4 mL of 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, containing FA methyl esters (FAMES) in n-heptane, was transferred to a sample bottle before analysis. The FA concentrations were determined via gas chromatography–mass spectrometry 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 using helium as the carrier gas, and an injection volume of 10 μL , injector temperature of 250°C, and initial temperature of 125°C; the oven temperature was increased to 145°C at 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 increased to 227°C at 1°C/min 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 FAMES was performed by comparison of retention times and full-scan EI mass spectra with those of a known concentration of 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 (SFAs, MUFAs, and PUFAs, respectively).

2.4 Statistical analysis

To explore differences among forms and areas of samples, a one-way analysis of variance (ANOVA) with a *post hoc* Tukey's honestly significant difference test was performed on the FA profiles and isotopic datasets. Stepwise discriminant analysis (SDA) was

performed to identify significant discriminatory variables (Rencher, 2002). A linear discriminant analysis was developed to discriminate between the two forms and between SCS and IO using the selected variables. Finally, leave-one-out cross-validation was used to determine the rate of correct classification for different forms and areas. All statistical tests were performed in SPSS version 19.0 (IBM Corp., Armonk, NY, USA) using a significance level of $p \leq 0.05$. Results are presented as mean value \pm standard deviation (SD).

3 Results

3.1 Stable isotopes

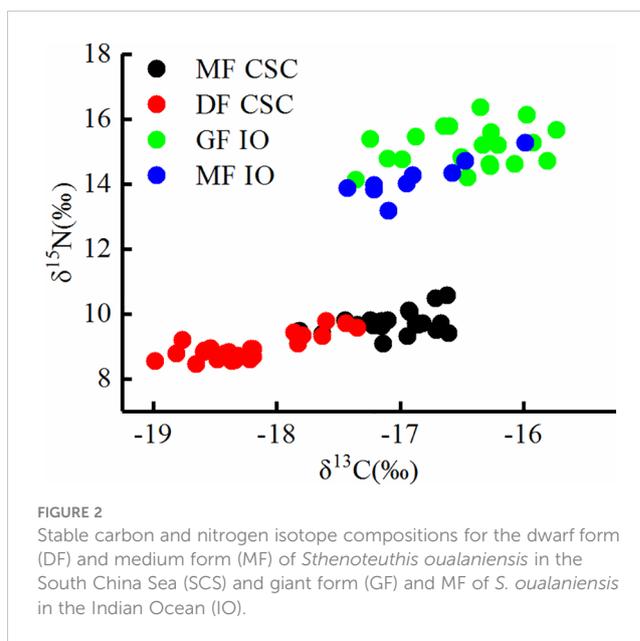
Analysis of *S. oualaniensis* from the SCS and IO identified significant differences for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\delta^{15}\text{N}$, $F = 891.783$, $P < 0.01$; $\delta^{13}\text{C}$, $F = 79.399$, $P < 0.01$). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of *S. oualaniensis* from the IO were higher than those of *S. oualaniensis* from the SCS (Table 2), indicating the geographic population differences. Similar results were obtained for isotopic values among forms. The MF from the SCS had higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values ($\delta^{15}\text{N}$, $F = 58.611$, $P < 0.01$; $\delta^{13}\text{C}$, $F = 117.829$, $P < 0.01$), and the GF from the IO had higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values ($\delta^{15}\text{N}$, $F = 16.272$, $P < 0.01$; $\delta^{13}\text{C}$, $F = 5.356$, $P < 0.05$). These observations indicate that isotopic values of *S. oualaniensis* differed not only between geographic populations but also between forms, although scatterplots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed considerable overlap between forms at the same sampling area (Figure 2).

3.2 FA profiles

A total of 24 FAs were identified in the muscle of *S. oualaniensis*, where PUFAs were the major FAs, accounting for 54.36%–62.73% of the total FAs, followed by SFAs and MUFAs (Table 3). The total content of SFAs in IO *S. oualaniensis* was significantly lower than that in SCS *S. oualaniensis* (ANOVA, $F = 5.672$, $P < 0.05$). Each SFA (except for 14:0 and 15:0) also exhibited a significant difference in the content between specimens from the SCS and IO. The total content of MUFAs in IO *S. oualaniensis* was significantly higher than that in SCS *S. oualaniensis* (ANOVA, $F = 58.963$, $P < 0.01$), and the content of most of the MUFAs differed between regions except for C16:1n7 and C18:1n9c. *S. oualaniensis* from the IO showed a higher content for most of PUFAs except for C20:5n3, C22:2n6, and C22:6n3 (Table 3). Among the PUFAs, both

TABLE 2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) for the dwarf form (DF) and medium form (MF) of *Sthenoteuthis oualaniensis* in the South China Sea and giant form (GF) and MF of *S. oualaniensis* in the Indian Ocean.

Form	$\delta^{15}\text{N}(\text{‰})$	$\delta^{13}\text{C}(\text{‰})$
DF CSC(N=25)	8.945 \pm 0.373	-18.237 \pm 0.429
MF CSC(N=24)	9.724 \pm 0.321	-17.056 \pm 0.304
MF IO(N=9)	14.161 \pm 0.562	-16.868 \pm 0.426
GF IO(N=21)	15.135 \pm 0.595	-16.448 \pm 0.446



22:6n3(DHA) and 20:5n3(EPA) were the major FAs and comprised >33% and 6% of the total FA content, respectively.

Principal component analysis (PCA) performed on the FAs revealed clear discriminations between forms as well as sampling regions (Figure 3). PC1 explained 51.0% of variance, and the highest contributions to PC1 were C20:0, C20:2, C24:1n9, C20:3n6, C17:0, and C18:2n6c (Table 4). PC2 yielded 19.1% of explainable results, with large contributions from C20:3n3, C22:2n6, and C20:4n6. Furthermore, PC3 explained 7.5% of variance with the most contributing descriptors being C14:0 and C18:1n9c (Table 4).

The separation between areas or forms was apparent in a three-dimensional plot (Figure 3). Thus, forms of *S. oualaniensis* could be effectively distinguished according to the PCA results, where the most significant distinction existed between GF and the other forms (Figure 3). Moreover, the results indicate that FA profiles, combined with PCA, can be used for authentication and traceability on the level of forms and areas of *S. oualaniensis* in the SCS and IO.

3.3 Combined analysis of stable isotope and FA compositions

Different forms and areas of *S. oualaniensis* may be identified through combined analysis of stable isotope and FA compositions as the analysis of a single stable isotope cannot effectively distinguish all forms in single area and the analysis of FAs cannot effectively distinguish all areas of origin. The results show that stable isotope compositions cannot be used as an indicator to distinguish between the DFs and MFs in the SCS or between the MFs and GFs in the IO. Meanwhile, FAs for the DF in the SCS and the MF in the IO partially overlap (Figure 3). Therefore, the combined use of stable isotope and FA markers can be a promising method to distinguish between different forms or origins of *S. oualaniensis*.

SDA was used to classify the four groups of *S. oualaniensis* by their different forms or origins according to the compositions of the

TABLE 3 Percentages (%) of total FAs for the dwarf form (DF) and medium form (MF) of *Sthenoteuthis oualaniensis* in the South China Sea and giant form (GF) and MF of *S. oualaniensis* in the Indian Ocean.

Fatty acid (%)	SCS		IO	
	DF	MF	MF	GF
C14:0	0.74 ± 0.09a	0.47 ± 0.06b	0.72 ± 0.18a	0.65 ± 0.15a
C15:0	0.55 ± 0.05a	0.44 ± 0.05b	0.56 ± 0.08a	0.45 ± 0.12b
C16:0**	21.49 ± 2.49ab	22.02 ± 2.05b	18.90 ± 6.70a	19.67 ± 2.85ab
C17:0**	1.68 ± 0.27a	1.66 ± 0.20a	2.31 ± 0.51b	2.17 ± 0.65b
C18:0**	6.59 ± 0.71a	7.74 ± 0.78a	7.74 ± 0.86a	12.23 ± 3.12b
C20:0**	0.13 ± 0.03a	0.11 ± 0.02a	0.20 ± 0.05b	0.16 ± 0.06b
ΣSFAs*	31.18 ± 2.12a	32.45 ± 1.85ab	30.43 ± 6.31a	35.33 ± 4.44b
C15:1n5**	0.10 ± 0.03a	0.09 ± 0.03a	0.14 ± 0.07b	0.11 ± 0.05ab
C16:1n7	0.39 ± 0.06ac	0.29 ± 0.06b	0.44 ± 0.09c	0.33 ± 0.10ab
C17:1n7**	0.28 ± 0.10a	0.23 ± 0.09a	0.46 ± 0.22b	0.31 ± 0.17a
C18:1n9t**	0.37 ± 0.13a	0.32 ± 0.10a	0.64 ± 0.20b	0.45 ± 0.19a
C18:1n9c	1.43 ± 0.12a	1.00 ± 0.14b	1.46 ± 0.27a	1.30 ± 0.38a
C20:1**	3.10 ± 0.41a	3.34 ± 0.32a	3.59 ± 0.85a	7.25 ± 2.55b
C24:1n9**	0.43 ± 0.13ab	0.40 ± 0.09b	0.73 ± 0.23c	0.55 ± 0.18b
ΣMUFAs**	6.09 ± 0.74ab	5.66 ± 0.34a	7.46 ± 1.27b	10.31 ± 3.31c
C18:2n6t**	0.82 ± 0.39a	0.71 ± 0.26a	1.66 ± 0.51c	1.20 ± 0.44b
C18:2n6c**	0.25 ± 0.06ab	0.21 ± 0.04a	0.38 ± 0.11c	0.29 ± 0.10b
C18:3n6**	0.29 ± 0.10a	0.24 ± 0.09a	0.53 ± 0.19b	0.32 ± 0.15a
C18:3n3**	0.29 ± 0.08a	0.25 ± 0.05a	0.39 ± 0.18b	0.34 ± 0.12ab
C20:2**	0.49 ± 0.12ab	0.44 ± 0.06a	0.72 ± 0.18c	0.58 ± 0.15b
C20:3n6**	0.36 ± 0.10ab	0.32 ± 0.07a	0.61 ± 0.19c	0.44 ± 0.15b
C20:3n3**	1.48 ± 0.59a	2.55 ± 0.29b	2.96 ± 0.75b	4.39 ± 0.85c
C20:4n6**	1.81 ± 0.28a	2.79 ± 0.32c	3.04 ± 0.83c	5.07 ± 1.03b
C20:5n3**	8.31 ± 0.37a	6.01 ± 0.83c	6.53 ± 0.87bc	6.67 ± 0.42b
C22:2n6*	2.91 ± 0.20a	2.38 ± 0.11b	2.77 ± 0.30a	2.35 ± 0.16b
C22:6n3**	45.72 ± 2.54a	45.34 ± 1.59a	42.50 ± 5.88a	33.38 ± 7.94b
ΣPUFAs**	62.73 ± 2.04a	61.89 ± 1.83a	62.11 ± 6.40a	54.36 ± 7.10b

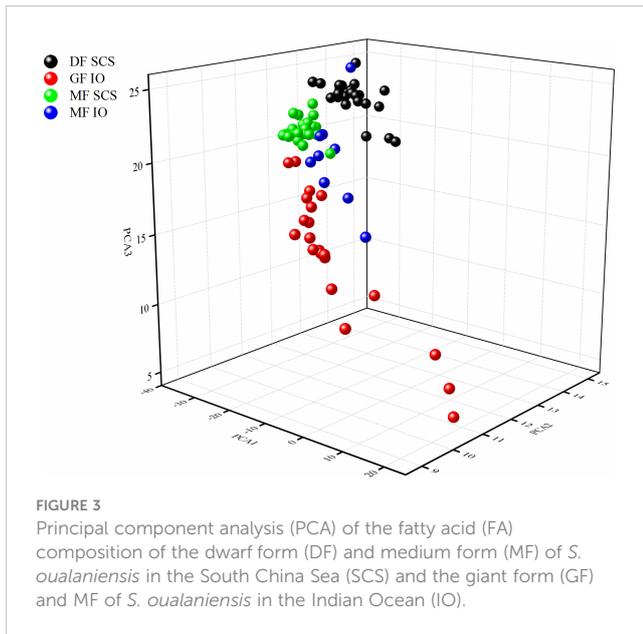
The symbol * indicates statistical significance at the $\alpha=0.05$ level.

The symbol ** denotes statistical significance at the $\alpha=0.01$ level.

Different lowercase letters within rows indicate significant differences ($P < 0.05$).

stable isotope and FA contents (Figure 4). Three discriminant functions were obtained after applying SDA. The variables selected by general statistics demonstrated that $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C20:4n6, and C20:5n3 contributed the most to the first discriminant function, whereas C22:6n3 and C20:5n3 contributed the most to the second and third discriminant functions, respectively (Table 5). A complete separation of the four groups

was achieved. The recognition ability of the discriminant functions for all groups was 100%, where the first, second and the third discriminant functions accounted for 91.3%, 7.3%, and 1.4%, respectively. The results indicate that $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and FA compositions, combined with SDA are promising effective methods for classifying and identifying the different forms of *S. oualaniensis* in the SCS and IO.



4 Discussion

4.1 Stable isotope ratios

The accumulation of stable isotopes in fish tissues is a comprehensive reflection of their complex physiological processes, encompassing their metabolism, growth, and feeding. Feeding is the primary source of exogenous isotopes and plays a particularly significant role in determining $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (MacNeil et al., 2005). In aquatic ecosystems, plankton, benthic algae, and certain aquatic and terrestrial higher plants (e.g., seagrass and mangroves) are the primary contributors to organic carbon. The carbon isotope ratios of consumers typically closely resemble those of their food sources. Since $\delta^{13}\text{C}$ exhibits a relatively low fractionation across trophic levels, this is often used to indicate the food sources of organisms (Doi et al., 2005). Nitrogen stable isotopes are limited in excretion during protein synthesis within organisms, producing higher $\delta^{15}\text{N}$ values in consumers compared with their food sources (Peterson and Fry, 1987). The trophic fractionation of $\delta^{15}\text{N}$ between adjacent trophic levels typically averages 3.4‰, which is crucial for indicating the trophic niche of fish species within the food chain (Yokoyama et al., 2005). Herein, we revealed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are both relatively high in *S. oualaniensis* from the IO, whereas $\delta^{13}\text{C}$ values from *S. oualaniensis* from the SCS exhibit a broader distribution range. This observation suggests that *S. oualaniensis* in the IO occupy higher trophic levels, whereas in the SCS, they have more diverse food sources. Such differences in distribution patterns are jointly determined by prey availability and geographical environments in different oceanic regions. Notably, the dietary composition significantly differed between *S. oualaniensis* from SCS and IO. In the IO, the prey mainly comprises fish, cephalopods, and crustaceans (Chen et al., 2007), whereas in the SCS, this primarily comprises large copepods, krill, amphipods, Myctophidae, and *Gonatus onyx* (Gong et al., 2016).

The two oceanic regions are geographically distant, producing notable disparities in environmental factors, including terrestrial inputs, precipitation, and temperature. These ecological variations impact the stable isotope composition of organisms as reported by Suh and Shin (2013). Specifically, the SCS, as a semi-enclosed basin, experiences diverse influences from terrestrial inputs and freshwater runoff. This diversity produces a wide array of basic prey species and an abundance of primary carbon sources, ultimately generating a broader distribution of $\delta^{13}\text{C}$ values among the fish fauna. The differences in $\delta^{15}\text{N}$ values primarily reflect the trophic positions of fish species within their respective food webs in the two oceanic regions. *S. oualaniensis* in the IO are characterized by larger body sizes and occupy higher trophic levels at the apex of the food chain and accumulate distinct nitrogen isotope signatures in their bodies. Furthermore, isotopic variations between the two regions are closely associated with primary productivity. In the open waters of the SCS, the existence of a thermocline obstructs the vertical mixing of seawater, impeding the replenishment of nitrogen and phosphorus from deeper, nutrient-rich layers to the euphotic zone. This limitation decreases the primary productivity (Shen et al., 2008), subsequently reducing the absolute isotopic values. Comparable observations have been documented in studies of *Dosidicus gigas*, where individuals inhabiting the oligotrophic waters off the coast of the middle east Pacific exhibit lower isotopic values than those found along the western coast of South America (Gong et al., 2018). The variations in stable isotope composition among fish species from different marine regions are attributable to multifactorial influences, including prey composition, geographical settings, terrestrial inputs, ecological conditions, and primary productivity. Collectively, these factors determine the positions and trophic niches occupied by fish species within the food chain, offering crucial scientific insights for the in-depth investigation of fish ecology, behavior, and resource distribution.

This study reveals differences in the isotope composition among various forms of *S. oualaniensis* within the same marine region. Specifically, larger-sized *S. oualaniensis* forms exhibited higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. These differences are closely related to the distinct trophic levels occupied by these forms within the ecosystem and the diversity of their food sources. According to food chain theory, predators prefer larger prey as they grow to maximize their nutrient intake. Therefore, larger *S. oualaniensis* forms are more inclined to prey on larger fish and cephalopods. This variation in feeding habits produces a diverse carbon source selection among the different forms, ultimately causing the differences in $\delta^{13}\text{C}$ values as reflected in their isotopic accumulation (Pitt et al., 2009). In addition to food sources, the stable isotope composition of organisms is influenced by their own metabolism. Biochemical processes involving carbon and nitrogen isotope fractionation impact the isotopic composition of organisms (Cui, 2012). Further research indicates that the habitat depth of *S. oualaniensis* gradually increases with their growth, and notable differences occur in the metabolic pathways of larger individuals. In the deep-sea regions of the IO, the GF can even use proteins as an energy source. Moreover, variations in life history among different forms can influence isotope accumulation. The metabolic effects associated with life history cannot be ignored in isotope analysis. Studies have revealed substantial differences in the life cycles of different forms of *S. oualaniensis*. For instance, the DF

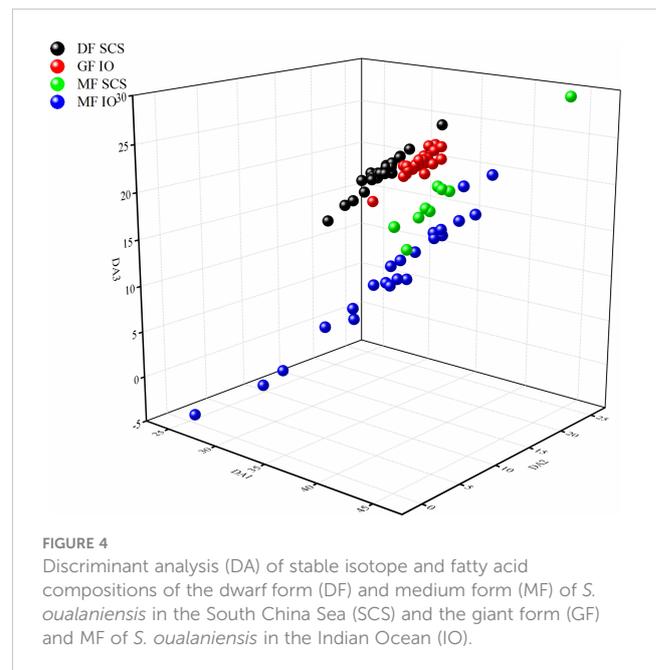
TABLE 4 Fatty acid (FA) contributions to principal component analysis (PCA) of dwarf form (DF) and medium form (MF) of *S. oualaniensis* in the South China Sea (SCS), and giant form (GF) and MF of *S. oualaniensis* in the Indian Ocean (IO).

Fatty acid	Principal component		
	1	2	3
C14:0	0.313	0.463	0.616
C15:0	0.682	0.635	0.093
C15:1n5	0.676	0.054	-0.018
C16:0	-0.327	0.044	-0.464
C16:1n7	0.766	0.559	0.013
C17:0	0.95	-0.087	-0.091
C17:1n7	0.761	0.183	-0.132
C18:0	0.627	-0.665	0.261
C18:1n9t	0.884	0.163	-0.197
C18:1n9c	0.468	0.395	0.555
C18:2n6t	0.877	0.038	-0.059
C18:2n6c	0.956	0.199	-0.156
C18:3n6	0.655	0.171	-0.216
C20:0	0.99	0.041	-0.055
C18:3n3	0.749	0.155	-0.055
C20:1	0.637	-0.627	0.379
C20:2	0.968	0.107	-0.081
C20:3n6	0.953	0.167	-0.212
C20:3n3	0.507	-0.771	0.095
C20:4n6	0.474	-0.793	0.174
C22:1n9	0.252	-0.277	0.492
C22:2n6	0.122	0.787	0.276
C20:5n3	-0.479	0.649	0.387
C24:1n9	0.964	0.095	-0.194
C22:6n3	-0.732	0.537	-0.127

in the SCS has the shortest life cycle of approximately 80 days (Jiang et al., 2019), whereas the MF has a life cycle of approximately 200 days (Lu et al., 2022). Conversely, the life cycles of the MF and GF in the IO exceed one year. During metabolic processes, heavier isotopes tend to accumulate in the body, and older individuals typically exhibit a heavier isotope composition (Malej et al., 1993; Overman and Parrish, 2001).

4.2 FA compositions

FAs, as crucial components of organisms, exhibit stability during energy transfer, making FAs a characteristic indicator of the feeding habits of a predator over a certain period (Rajendran et al., 1993; Stübing and Hagen, 2003). The current study revealed discrepancies in



the FA composition of *S. oualaniensis* from different sea areas, which concurs with the isotopic analysis results. Notably, the content of C17:0 in individuals from the IO was consistently higher than that of individuals from the SCS, which is potentially linked to the distinct ecological systems of these two regions. Such differences in feeding preferences among *S. oualaniensis* across various sea areas have been observed in other marine habitats. In further exploring these disparities, we hypothesized that the variations in FA composition may reflect adaptations to the unique trophic environments of each sea area. The higher concentration of C17:0 in the IO suggests a potential preference for prey species that are rich in this particular FA. Conversely, the lower levels in the SCS may indicate a different dietary profile, possibly because of the availability of different prey species or trophic niches. Understanding these feeding preferences is crucial in elucidating the trophic interactions and ecological roles of *S. oualaniensis* within diverse marine ecosystems.

We applied PCA and observed that the distribution of the same form across different sea areas was closer than the distribution distances between different forms. This suggests a degree of similarity in feeding strategies among MF individuals in the IO and the SCS. Consistent with this, the isotopic $\delta^{13}\text{C}$ values showed considerable overlap among MF individuals in these two sea areas. Additionally, the FA composition of *S. oualaniensis* from different forms notably differed. The food composition of individuals of different forms varies because of size-related differences. DF individuals in the SCS exhibit a feeding preference for nektonic and planktonic organisms (Gong et al., 2018) and primarily feed on cephalopods, followed by crustaceans such as shrimps and copepods (Xie et al., 2021). Conversely, MF individuals have a nektonic feeding type (Gong et al., 2016), and primarily consume cephalopods and fish (Xie et al., 2021). In the northwestern IO, the MF primarily feeds on fish (62.4%) and cephalopods (35.8%) (Chembian and Mathew, 2014), whereas the GF mainly feeds on myctophids. The distribution of FA compositions within the two forms in the SCS exhibits a smaller overlap compared with that between the

TABLE 5 Contribution of fatty acid (FA) and stable isotopes to discriminant functions of dwarf form (DF) and medium form (MF) of *S. oualaniensis* in the South China Sea (SCS) as well as giant form (GF) and MF in the Indian Ocean (IO).

Characters	Discriminant functions		
	1	2	3
C18:1n9c	0.513	-0.502	0.209
C18:3n6	-0.510	-0.085	0.266
C20:1	-0.615	0.580	-0.442
C20:4n6	0.900	0.607	-0.074
C22:2n6	0.009	-0.611	0.606
C20:5n3	-0.890	-0.477	-1.062
C22:6n3	0.449	0.730	0.755
$\delta^{15}\text{N}$	1.333	-0.463	-0.028
$\delta^{13}\text{C}$	-0.377	0.497	0.322

two forms in the IO, potentially because of a higher similarity in feeding habits among the IO forms. Regardless of whether they live in the SCS or IO, GF individuals possess a higher content of SFAs than DF individuals, with a particularly prominent trend observed in the IO. This could be attributed to the role of SFAs in energy supply as larger individuals require a greater energetic input. Higher concentrations of C15:0 in the GFs across both oceans may be linked to varying feeding strategies among different forms. Additionally, the FA analysis suggests a preference for algae among *S. oualaniensis*. DF individuals in both oceans exhibit higher levels of C16:1n7 and C18:1n9, whereas those in the SCS also contain a higher amount of EPA. However, no significant difference in the EPA content was observed between the GF and MF individuals in the IO. The presence of C20:4n6, an indicator of benthic feeding, was higher in individuals from the IO than in those from the SCS. The larger-sized form in both oceans also demonstrated higher levels of C20:4n6, which was potentially related to the differing depths of their habitats as larger individuals often inhabit deeper water layers (Chesalin and Zuyev, 2002). The high content of DHA and EPA in *S. oualaniensis* from the SCS allows for the analysis of trophic levels through the DHA/EPA ratio. Our study reveals a trophic level hierarchy: the MF in the SCS > the MF in the IO > the DF in the SCS > the GF in the IO. This trophic ranking based on DHA/EPA differs from that obtained through isotopic analysis. Although deep-sea fish obtain DHA from consuming algae, the DHA in *S. oualaniensis* is probably accumulated through the predation of deep-sea fish. Notably, the DHA content in the GF of the IO was significantly lower than that in both SCS forms and the MF of the IO, potentially because of a stronger preference for squid in their diet, which subsequently reduces the accumulation of DHA in their bodies.

4.3 SDA using isotope ratios and FA compositions

Abundant studies on cephalopod form classification have primarily focused on morphological and molecular perspectives. However, this

study innovatively classified geographical and form groups from a feeding ecology angle, and this dietary-based approach demonstrates a significantly higher accuracy than morphological-based methods. For instance, using the SDA method, the final correct cross-validated classification rates ranged from 44.3% to 75.2% among the four geographic regions (north-western Indian Ocean, Tropical Eastern Pacific, Bashi Channel and SCS) based on the beak morphology (Liu et al., 2018). Conversely, discrimination accuracy based on external morphology for different groups of *S. oualaniensis* in the Bashi Channel and the SCS is 68.2% (Wang et al., 2019). Researchers have posited that discrimination accuracy using a single method is lower than that achieved by combining multiple methods. However, combining two standardized hard structures increases the correct classification of SDA by nearly 20% compared with using only one structural element (Fang et al., 2014). Consistent with our findings, isotopic composition distinguishes individuals from different sea areas but not from distinct forms within the same sea area. Conversely, FA composition differentiates between forms in the same sea area but not between individuals from separate sea areas. However, combining isotopic and FA methods facilitates accurate discrimination of *S. oualaniensis* from diverse sea areas and forms, achieving a discrimination accuracy of 100%. This is attributable to significant carbon source variations among *S. oualaniensis* from different sea areas, whereas those from the same region may overlap in feeding patterns and ecological niches influenced by factors such as growth. Distinct feeding strategies among various forms in the same sea area produce differing FA profiles, whereas similar feeding preferences among forms across sea areas result in some overlap in their FA composition. These disparities between sea areas and forms are ultimately reflected in variations of isotopic and FA profiles. Notably, isotopic composition plays a more pivotal role than FA composition in discriminating Atlantic salmon from distinct regions (Thomas et al., 2008). This underscores the significance of an integrated approach that merges isotopic and FA analyses for precise cephalopod form discrimination. Although employing isotopic or FA methods separately can yield valuable insights, their combined application offers a more comprehensive understanding of cephalopod form structures and ecologies. This interdisciplinary approach, integrating biochemical and ecological perspectives, is promising for future investigations aiming to elucidate the intricate relationships among cephalopod feeding ecologies, population dynamics, and their environmental habitats. This method exhibits high practicality and can function as an efficient tool for monitoring the source of *S. oualaniensis* on the market, thereby guaranteeing its traceability. Furthermore, the study has revealed significant variations in food sources and ecological niches across diverse geographical regions and forms of *S. oualaniensis*, thus offering crucial insights for fisheries management. More specifically, by leveraging these differences, fisheries managers can formulate more targeted management strategies, aiming to foster sustainable exploitation of distinct sea areas and forms, and concurrently minimize the hazards linked to overfishing.

In conclusion, this study measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as well as the FA content in samples from distinct forms of *S. oualaniensis* in two marine regions. We conducted a thorough analysis of feeding and trophic ecological disparities among these groups, followed by SDA. The findings indicated that individuals from the IO occupied higher trophic levels, whereas those from the SCS exhibited a more diverse

dietary range. Variations in $\delta^{13}\text{C}$ distributions among individuals from the two marine regions were jointly influenced by feeding preferences and geographical conditions. Conversely, the discrepancies in $\delta^{15}\text{N}$ values were primarily attributed to the trophic positions of individuals within their respective food webs. Forms with larger body sizes tended to possess higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, reflecting their occupation of distinct trophic levels and dietary sources within the ecosystem. Isotopic composition effectively distinguished individuals from different marine regions, whereas FA composition discriminated among forms within the same marine region. Therefore, a combined approach using both isotopes and FAs enabled the accurate discrimination of *S. oualaniensis* from various marine regions and forms. This study utilized samples of *S. oualaniensis* collected from the SCS and the IO. While these two regions possess a certain level of representativeness, they do not fully encapsulate the entire distribution range of the species. To further investigate the morphological variations and source diversity of *S. oualaniensis*, future research efforts could expand sample collection to encompass a broader geographical scope, including other oceanic regions and diverse ecological environments. Such an approach is anticipated to significantly enhance the generality and precision of research findings.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors. Requests to access these datasets should be directed to Kai Zhu zhukaild@126.com.

Author contributions

KZ: Writing – original draft. KX: Writing – review & editing. WZ: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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