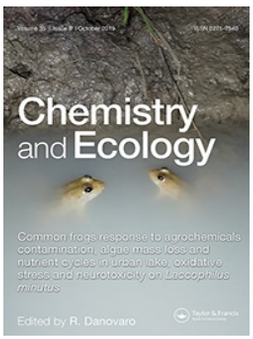

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$\delta^{13}C$ and fatty acid composition of mesopelagic fishes in the South China Sea and their influence factors

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ABSTRACT

Study of the ecology of mesopelagic fishes is central for assessing the active biological pump in the ocean, especially in the mesopelagic layers. The use of $\delta^{13}C$ and fatty acid analysis can help to analysis the ecology of mesopelagic fishes. Here, we analysed the fatty acid composition of mesopelagic fishes from the continental northern slope of the South China Sea (NSSCS) and compared with nearshore SCS fishes and mesopelagic fishes collected from the Southern Ocean. The mesopelagic fishes had unusually high lipids, which resulted in $\Delta\delta^{13}C$ values exceeding 1‰, more than the enrichment factor in the food web. The mesopelagic fishes had higher C18:1n-9/C18:1n-7 and C20:1n-9/C18:1n-7 ratios compared with other fishes in the SCS, which confirmed that plankton were their main dietary source. The mesopelagic fishes from SCS and Southern Ocean had different ratios of C20:5n-3/C22:6n-3 (EPA/DHA), suggesting geographical locations and diet sources had obvious influence on their fatty acid composition. The SCS mesopelagic fishes had higher C20:4n-6/C22:6n-3 (ARA/DHA) and C20:4n-6/C20:5n-3 (ARA/EPA) ratios than mesopelagic fishes in the Southern Ocean, indicating the influence of physical factors on fatty acid composition. Thus, future studies of the fatty acids in mesopelagic fishes should consider both dietary sources and physical environments.

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Mesopelagic fishes; lipids; $\delta^{13}C$; fatty acids; temperature; South China Sea

Introduction

The rapid increase in demand for high-quality protein poses great challenges on global commercial marine fisheries [1], stimulating search for alternative fishery resources such as mesopelagic fishes, which are distributed worldwide [2,3]. Mesopelagic fishes comprise a biomass of more than 10 billion tons and constitute a potential solution to the demand for high-quality protein [4,5]. Most mesopelagic fishes perform diel vertical migration (DVM) by migrating upward into the epipelagic zone at night and returning to the mesopelagic zone during the daytime [2,6,7]. Accordingly, mesopelagic fishes link primary

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consumers including copepods and zooplankton, and higher predators including large pelagic fishes, benthic fishes, and marine mammals [8,9,10]. Consequently, their DVM behaviour plays an important role in ocean food webs [4,11] through transfer of organic matter from the upper productive layer to deeper layers [12–14]. However, the large biomass of mesopelagic fishes is underutilised due to inadequate knowledge of their ecological characteristics. This knowledge is required in order to understand the role played by mesopelagic fishes in biological pump, and their sustainable exploitation in the future.

To understand the DVM behaviour of mesopelagic fishes, studies using fatty acid biomarkers and stable isotope ($\delta^{13}C$, $\delta^{15}N$) are required because traditional stomach content analysis does not reflect long-term feeding behaviour and readily degradable material in the diet can be underestimated [15]. Fatty acid biomarkers and stable isotope methods can overcome the disadvantages of stomach content analysis. These methods have been used successfully in fish studies to identify dietary sources at different trophic levels [16–18]. The usefulness of many fatty acids as biomarkers of dietary sources is attributed to their limited biosynthesis ability to only certain bacteria, phytoplankton and macroalgal species in the marine environment [19,20]. However, a few studies have suggested that variations in lipids composition might mask the dietary sources revealed by $\delta^{13}C$ and physical environmental factors such as temperature may affect the fatty acid composition of mesopelagic fishes [19,21,22]. Moreover, extreme environmental conditions such as wide variations in temperature may effect fishes physiology because they must change their fatty acid composition to maintain the fluidity of their cell membranes [23,24]. Notably, highly unsaturated fatty acids (HUFAs; FAs having $\geq$20 carbon atoms and $\geq$3 double bonds) play important structural and functional roles in adaptation to environmental stressors [24]. Apart from fatty acids, the $\delta^{13}C$ provides a chemical record of primary production sources in higher trophic consumers [25,26]. The $\delta^{13}C$ is more depleted during lipids synthesis than proteins and carbohydrates biosynthesis [27,28]. Interestingly, most mesopelagic fishes have a higher lipid content than other fishes due to their DVM [22,29]. Thus, the high lipid content in mesopelagic fishes can mask the dietary $\delta^{13}C$, making dietary reconstructions difficult [30,31]. However, knowledge of the factors affecting $\delta^{13}C$ and fatty acid composition on mesopelagic fishes is rarely studied.

The South China Sea (SCS) is the largest semi-closed sea in the western tropical Pacific Ocean and the second largest marginal sea worldwide [32,33]. The physical environment of the northern slope of the South China Sea (NSSCS) is complex [32,34,35], providing macro habitats for approximately 1100 fish species dominated by mesopelagic fishes [36]. Despite their dominance, our knowledge on the ecology of mesopelagic species in the NSSCS is currently limited. Understanding the fatty acid composition and diets of mesopelagic species provides useful knowledge on their contribution to the active biological pump.

In this study, we hypothesised that the high lipid content in mesopelagic fishes masks the dietary $\delta^{13}C$. We further assumed that the diet sources and physical environmental factors control the fatty acid composition of mesopelagic fishes. To test these hypotheses, we investigated the mesopelagic fishes from the NSSCS by using fatty acids and stable isotope analyses. In particular, we studied the impact of lipid content on the $\delta^{13}C$ of mesopelagic fishes. The potential factors controlling the composition of fatty acids were also

evaluated by comparing the samples collected from the near-shore region of the SCS and the Southern Ocean.

Materials and methods

Sampling

Fish samples were collected from the continental slope of the SCS (stations L1, L2, L3 and L4) during a cruise carried out in October 2014 (R/V Nan Feng) (Figure 1). The sampling of the nearshore SCS station L5 (Figure 1) was carried out in May 2011 using a local fishing boat. The fishes from the continental shelf areas of the SCS (stations L1 and L4) were caught by using a bottom trawl having a 150 m mouth perimeter and a 51.5 m head rope length. Fishes from the deep slope of the SCS (stations L2 and L3) were caught by using a mid-layer trawl having a 136.1 m mouth perimeter and a 30.0 m headope length. The sampling dates, locations, and water depths for stations L1 to L4 are listed in Table 1.

Preliminary catch analysis was performed on-board depending on the size. For total catches with weight less than 5 kg, all specimens were identified, counted, measured, and recorded. For larger catches with more than 5 kg, all obvious large specimens and

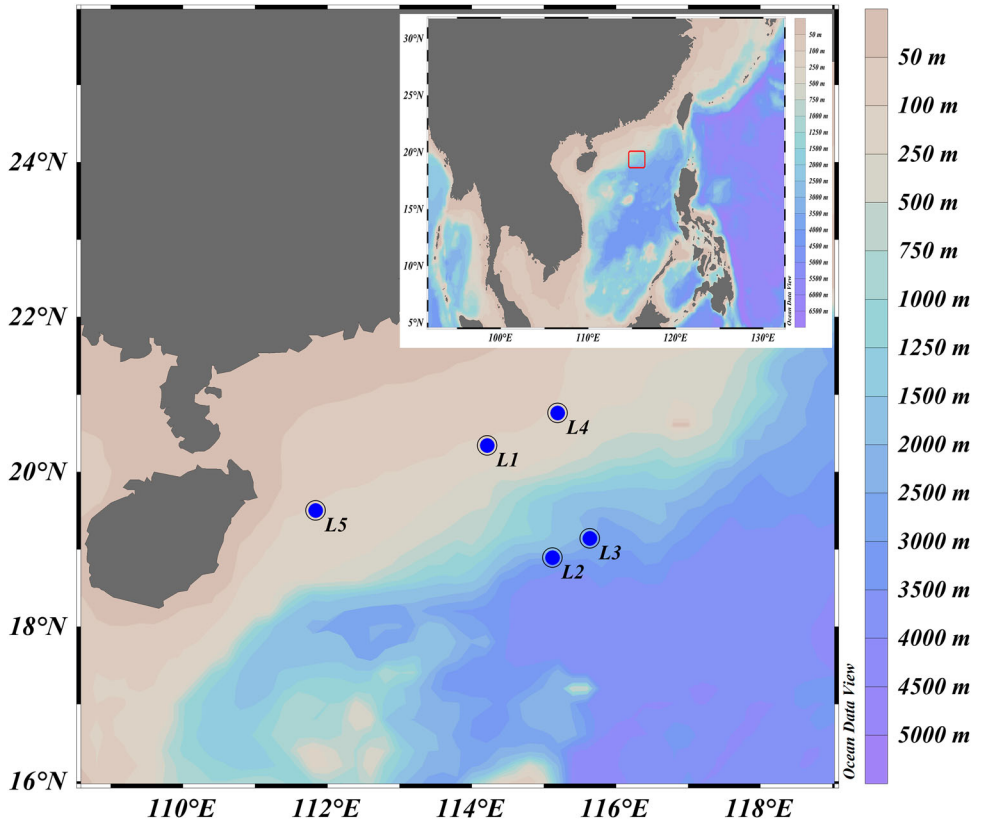

Figure 1. Map of sample collection locations.

Table 1. Sampling locations, dates, depths and fish species in the South China Sea.

Station	Longitude	Latitude	Date 2014	Time	Bottom depth (m)	Sample depth (m)	Species
L1	114°09′30″E	20°17′10″N	10/13	14:00–17:00	120	120	*Nemipterus bathybius, Todarodes pacificus, Wart perch, Loligo duvaucelii, Decapterus macrosoma, Red Seabream*
L2	115°05′23″E	18°55′56″N	10/14	14:55–17:48	3000	380	*Diaphus luetkeni, Diplophos taenia, Cubiceps natalensis, Lampanyctus niger*
L3	115°31′53″E	19°01′11″N	10/20	0:30–3:30	2210	430	*Myctophum asperum, Myctophum obtusirostre, Lampanyctus niger*
L4	115°01′22″E	20°43′01″N	10/16	14:44–17:07	120	120	*Apogon semilineatus, Priacanthus macracanthus, Decapterus maruadsi, Sepia esculenta, Upeneus bensasi, Champsodon atridorsalis*

rare species were removed and the remaining catch was subsampled proportional to its weight for further processing [37]. The identification of each species was done by using morphological characteristics and fish taxonomic guides [38–42]. Fishes from the continental shelf areas of the SCS were considered SCS epipelagic fishes, based on their non-migration. Fishes from the deep slope of the SCS were considered SCS mesopelagic fishes because of their DVM behaviour in the deep slope of the SCS [37]. Plankton samples were collected by vertical trawling using a net having mesh sizes of 76, 167, and 505 µm. The plankton samples were washed using filtered seawater, and filtered onto pre-combusted (450°C, 5 h) 47 mm GF/F filters and preserved at −20°C freezer for future analysis.

All the fish samples were stored frozen at −20°C until transferred to the State Key Laboratory of Estuarine and Coastal Research of the East China Normal University, Shanghai, China for further analysis. In the laboratory, fish species of similar size were chosen for further analysis (Appendix 1). The skin and scales were removed from each fish and muscle tissue was excised from below the dorsal fin. The muscle tissue samples were lyophilised in a freeze dryer (LOC-1; Christ, Germany) and stored at −40°C until needed for analysis [17]. The dried muscle samples were ground by using a mortar and pestle.

Fatty acids analysis

The fatty acid composition of plankton and fishes was determined from a known quantity of tissue extracted using a dichloromethane–methanol solvent system (2:1 v/v, using 0.01% butylated hydroxytoluene, BHT), based on the Folch method for total lipid determination [18,43]. A 100 mg of dorsal muscle samples was mixed with approximately 15 mL of a mixture of dichloromethane and methanol (2:1). The mixture was extracted and centrifuged (3000 rpm, 10 min), and the supernatant was transferred to a flask using a pipette. The solvent was evaporated to dryness under a stream of N_2 at room temperature, weighed, and the lipid content was calculated as the weight percent of the unextracted freeze-dried tissue [31].

The fatty acids were transformed to fatty acid methyl esters (FAMEs) by using a mixture of methanol (containing 5% HCl) and n-hexane and held at 50°C for approximately 12 h [44]. The FAMEs were analysed by using a gas chromatography mass spectrometry

(7890A; Agilent, USA) equipped with a DB-FFAP capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness; Agilent, USA). During analysis, C21:0 and C19:0 methyl ester were added to the samples as internal recovery and quantification standards, respectively. The injector and detector temperatures were both 250°C. The injections (1 μl) were made at 60°C and the temperature was increased to 170°C at a rate of 30°C/min. The temperature was held constant for 5 min, then increased to 220°C at 1°C/min, and held at this temperature for 10 min. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. FAMEs were identified by comparison of retention times with commercial standards (37 Component FAME Mix; Supelco^{TM}). The contents of particular fatty acids were expressed as the relative percentage of the total fatty acids contents based on peak areas. The fatty acids recovery rate in the analysis was >80%.

Stable isotope analysis

The extracted muscle tissue was dried under a stream of liquid N_2 at room temperature and subsequently used for $\delta^{13}C$ measurement. Stable carbon isotope ratios ($\delta^{13}C$) were measured for tissue before lipid extraction (unextracted tissue; $\delta^{13}C_{bulk}$) and after extraction (extracted tissue; $\delta^{13}C_{extracted}$). Dried and ground samples were weighed into tin cups for ^{13}C and ^{15}N analysis. Stable carbon and nitrogen isotopes were measured by using an isotope ratio mass spectrometer (Finnegan Delt plus XP; Thermo, Germany). The results were normalised to Vienna Pee Dee Belemnite standard (PDB) for $\delta^{13}C$ [15]. The stable isotope ratios are expressed in δ notation of units per mill as follows [28]

$$\delta X(‰) = ((R_{sample}/R_{standard})-1) \times 1000, \quad \text{where } X = {}^{13}C \text{ and } R = {}^{13}C/{}^{12}C$$

The precision of the stable isotope analyses was ±0.1‰. The C:N ratio was expressed as a molar ratio.

Statistical analysis

Results are reported as the mean ± the standard deviation (SD) whenever more than one sample was analysed. The data were tested for normality by using Shapiro–Wilk test and homogeneity of variance using Levene's test. Data with unequal variances were transformed (arcsine of the square root) to ensure homogeneity of variances [45]. For $\delta^{13}C$ values, paired comparisons were made between lipid extracted tissue ($\delta^{13}C_{extracted}$) and unextracted tissue ($\delta^{13}C_{bulk}$) by using paired *t*-test. One-way analysis of variance (ANOVA) was used to test for significant differences in fatty acids composition and dietary sources among of mesopelagic fishes from the NSSCS, nearshore SCS and mesopelagic fishes from the Southern Ocean. The Spearman correlation was used to examine the relationship between lipid content, C:N ratio, and $\Delta\delta^{13}C$ values (i.e. the difference in $\delta^{13}C$ value between unextracted and extracted tissue). A cluster analysis based on fatty acids was performed using PRIMER 5.0 [46]. Principal component analysis (PCA) was used to investigate variation in the fatty acid signatures among the fish species, and to identify the most fatty acids responsible for the variations [22]. Results with *p* values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS for windows version 23 (SPSS version 23, IBM, Armonk, NY, USA).

Results

Fish lipid content, C:N ratio and $\Delta\delta^{13}C$

In this study, a total of 77 fishes were analysed from SCS as listed in Table 1. Based on their migration, feeding habits and living environment, these fishes were classified as nearshore fishes, epipelagic fishes and mesopelagic fishes (Appendix 1). Briefly, the nearshore fishes live in the coastal and river estuary areas feeding on copepods and detritus. The epipelagic fishes mainly live in the continental shelf areas feeding on plankton and detritus. The mesopelagic fishes live in the deep slope areas feeding on zooplankton from the surface layers at night and migrate to the mesopelagic layers during the daytime.

The lipid content, C:N ratio and $\Delta\delta^{13}C$ values of epipelagic and mesopelagic fishes are listed in Appendix 1. The mesopelagic fishes had a higher lipid content, C:N, and $\Delta\delta^{13}C$ than the epipelagic fishes ($p < 0.01$). Following lipid extraction, the $\delta^{13}C$ values for the mesopelagic fishes changed larger than epipelagic fishes (Appendix 1). Significant positive relationships were obtained between the lipid content and the C:N ratio ($R^2 = 0.88$; $p < 0.001$), the C:N ratio and $\Delta\delta^{13}C$ ($R^2 = 0.84$; $p < 0.001$), and the lipid content and $\Delta\delta^{13}C$ ($R^2 = 0.83$; $p < 0.001$) (Figure 2).

The fatty acid composition of fishes and plankton from SCS

The fishes from the SCS varied in their fatty acid composition (Appendix). For most fishes, polyunsaturated fatty acids (PUFAs) were the major compounds, accounting for 30% to 60% of the total fatty acid content. The saturated fatty acid (SFAs) content did not vary substantially amongst all fishes. Of the fatty acids identified, C16:0, C18:0, C18:1n-9, C20:4n-6, C20:5n-3, and C22:6n-3 comprised 90% of the total fatty acids content, with other fatty acids typically comprising <3%.

The PCA results showed clear separation of the fatty acid signatures of the fishes and plankton (Figure 3). The PCA identified five main groups, with components PC1 and PC2 accounting for 55.4% of the variations. The plankton and fishes were separated into two distinct groups. Based on factor loading, PC1 comprised mainly the fatty acids C14:0, C18:4n-3, C15:0, C18:3n-3, and C22:6n-3, while PC2 comprised mainly C18:1n-7, C20:1n-9, and C18:1n-9. The plankton had higher percentages of C14:0, C18:4n-3, C15:0, and C18:3n-3 fatty acids than the fishes (Appendix 1; $p < 0.01$). The content of C14:0 in plankton ranged from 6% to 14%, compared with between 0.7% and 4% for fishes. In addition, the C18:4n-3, C15:0, and C18:3n-3 contents in plankton were >1%, compared with <1% in fishes ($p < 0.05$). On the contrary, the fishes had more C22:6n-3 than the plankton. The SCS mesopelagic fishes had the highest C18:1n-9/C18:1n-7 and C20:1n-9/ C18:1n-7 ratios, while the nearshore fishes had the lowest ratios and the SCS epipelagic fishes had intermediate values (Figure 4).

The fatty acids composition of mesopelagic fishes and plankton from SCS and Southern Ocean

The SCS mesopelagic fishes had stable SFA contents. The PUFAs were the major compounds, accounting for 30% to 54% of the total fatty acids content. Of the fatty acids identified, C16:0, C18:0, C18:1n-9, C20:4n-6, C20:5n-3, and C22:6n-3 made up ~90% of the total

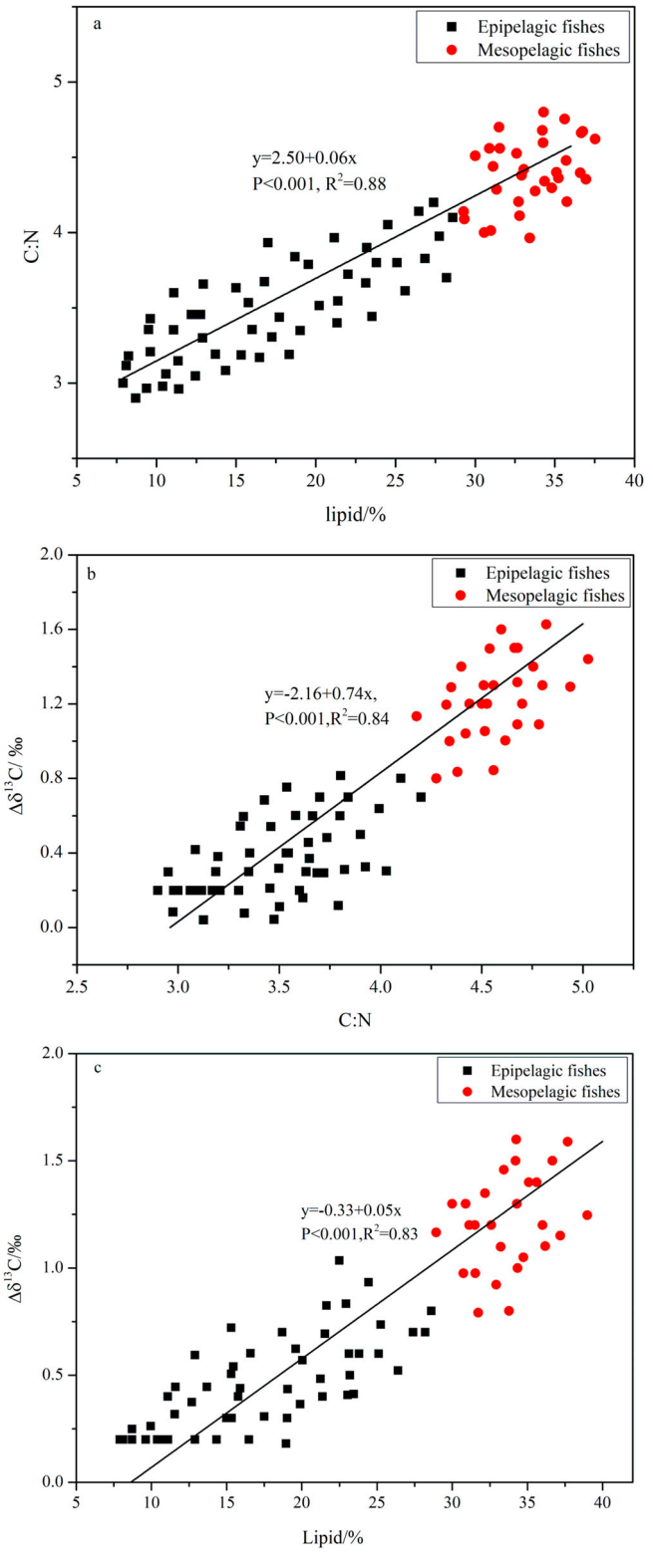

Figure 2. The relationships between (a) lipid content and the C:N ratio, (b) the C:N ratio and $\Delta\delta^{13}C$, and (c) the lipid content and $\Delta\delta^{13}C$ of fishes from the SCS.

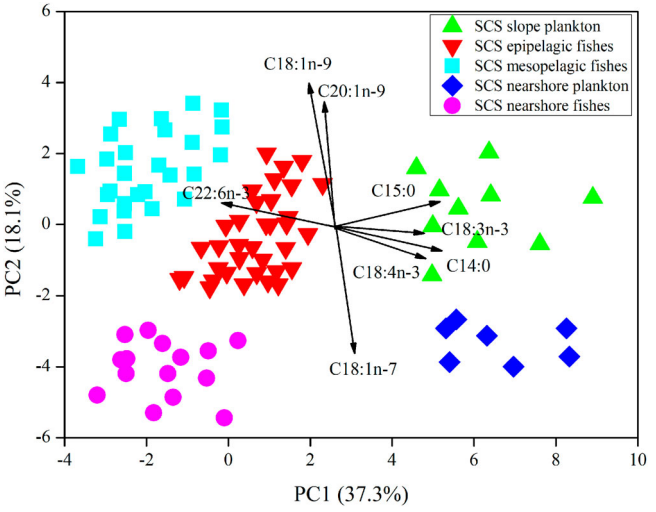

Figure 3. The principal component analysis of fatty acids of plankton and fishes from the SCS.

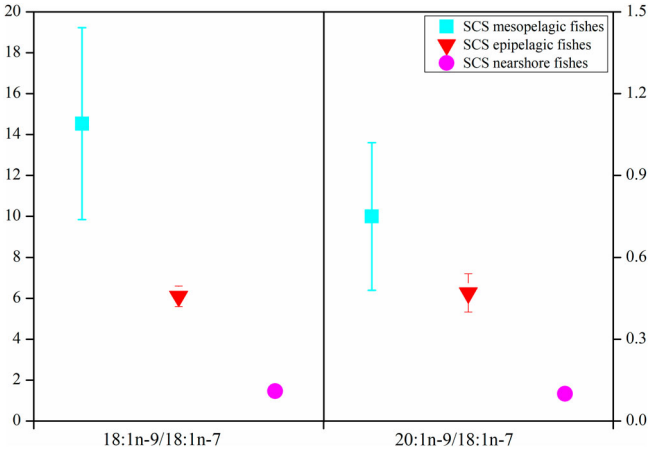

Figure 4. The fatty acids ratios of SCS fishes.

fatty acids content. The remaining fatty acids typically comprised <3%. Based on the PCA, the mesopelagic fishes and plankton from the SCS and the Southern Ocean were separated into four groups based on fatty acid content (Figure 5). The PC1 separated the groups into different oceanic regions, while PC2 separated them into plankton and mesopelagic fishes. Based on factor loading, the C22:6n-3, C16:0, C18:1n-7, C20:4n-6 and C20:1n-9 fatty acids contributed mainly to PC1, while the C14:0 and C20:5n-3 fatty acids contributed mostly to PC2. The ratios of the major fatty acids were calculated based on the PCA results. The plankton and mesopelagic fishes from the Southern Ocean had higher C20:5n-3/C22:6n-3 (EPA/DHA) ratios, and the EPA/DHA ratio in Southern Ocean plankton was larger than 1. The plankton and mesopelagic fishes from the SCS had higher C20:4n-6/C22:6n-3 (ARA/DHA) and C20:4n-6/C20:5n-3 (ARA/EPA) ratios than

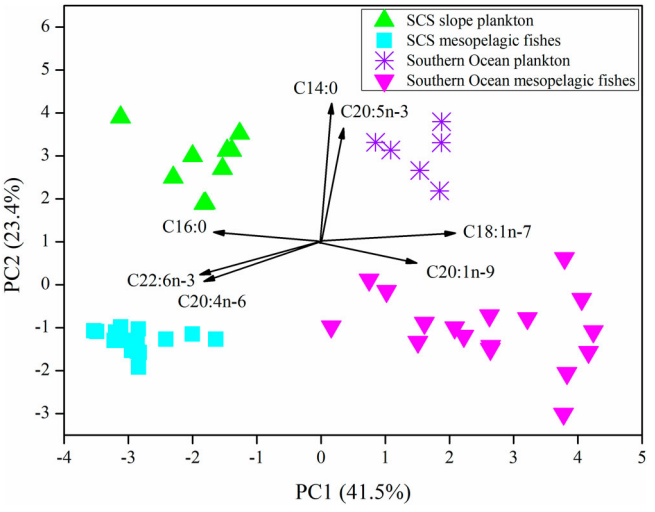

Figure 5. The principal component analysis of fatty acids in plankton and mesopelagic fishes.

those from the Southern Ocean ($p < 0.01$). The ARA/EPA ratio in SCS mesopelagic fishes were several times higher than that in the SCS plankton (Figure 6).

Discussion

We investigated the fatty acids and stable isotopes of mesopelagic fishes from the NSSCS and assessed their potential factors. We were interested in the mesopelagic fishes because of their important role in the active biological pump in the ocean. Their special DVM behaviours cause higher lipids in mesopelagic fishes than other fishes. Fatty acids can be used as biomarkers of dietary sources in fishes [47]. However, the fatty acid composition of

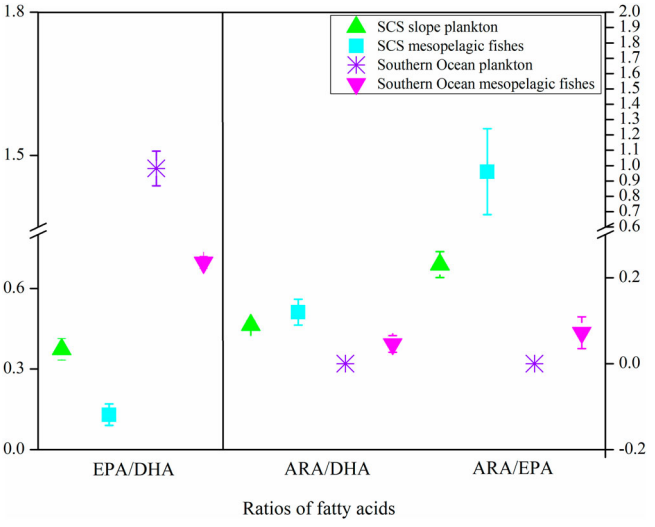

Figure 6. The fatty acids ratios of plankton and mesopelagic fishes from the SCS and Southern Ocean.

mesopelagic fishes may be affected by physical environmental factors such as temperature. We discuss the factors affecting the $\delta^{13}C$ and the fatty acids composition in mesopelagic fishes.

Lipid content impacted the $\delta^{13}C$ in mesopelagic fishes

It is usually known that $\delta^{13}C$ is moderately enriched (<1‰) during trophic transfer in food chain studies [28,48]. Therefore, $\delta^{13}C$ analysis provides information regarding the sources of primary production in the ecosystem [25]. In the present study, the lipid content of mesopelagic fishes was >30%, similar to the content reported for mesopelagic fishes of the Southern Ocean, but higher than that reported for freshwater and coastal fishes [19,49]. A high lipid content-enabled mesopelagic fishes to easily adapt to DVM and supply energy in the mesopelagic layers [2,6,7]. Thus, the high lipid content played an important role in the migration of mesopelagic fishes and is a unique characteristic of such fishes. Meanwhile, the $\Delta\delta^{13}C$ values caused by the high lipids in mesopelagic fishes exceeded the typical level of $\delta^{13}C$ enrichment in the food chain. Comparison of trophic levels based on $\delta^{13}C$ values showed that carbon discrimination leads to the differences between mesopelagic fishes and species with a low lipid content, including epipelagic fishes. The unextracted $\delta^{13}C$ did not accurately reflect the differences caused by various dietary sources. Hence, $\delta^{13}C$ values based on lipid content should be used in studies investigating the trophic structure of mesopelagic species.

The C:N ratio is controlled by the molecular structure of protein and lipid [30,31]. Therefore, its value might reflect the lipid content of fishes to some extent. In our study, the impact of lipids is ignored, when the lipid content of fish tissues was <17% (C:N ratio was <3.5), which is consistent with other results [30,48]. However, the high lipid content in mesopelagic fishes could have a substantial impact on analysis. Accordingly, when established methods are used to study the dietary sources of mesopelagic fishes, erroneous results are inevitable. Consequently, lipid correction is particularly important in the analysis of mesopelagic fishes. Indeed, a mathematical normalisation formula has been used widely in the ocean ecosystem [30]. However, our results showed different values compared to previous studies [30,49]. These differences are caused by many factors as follows. First, different phytoplankton, macroalgae, zooplankton or bacteria have their own fatty acids composition [50–54]. In most cases, the $\delta^{13}C$ of unsaturated fatty acids is depleted relative to saturated fatty acids, thus, the $\delta^{13}C$ may be impacted by the relative contribution of fatty acids from different diet sources [55]. Secondly, lipid content might be related to life-stage, breeding status, foraging ecology, season and geographic location [56,57] such that multiple factors may cause discrepancies among different studies [30,49,58]. Therefore, more detailed studies on mathematical normalisation formula for mesopelagic fishes should be conducted in the future.

Geographical location affected the fatty acid composition of SCS fishes

Fatty acids and fatty acid ratios are used as biomarkers of different food sources, and they can indicate the relative importance of one food source over another. The C18:1n-7 fatty acid has been used as a biomarker for sediment and suspended particulate matter, while the C18:1n-9 and C20:1n-9 fatty acids have been used in studies of plankton and

zooplankton, respectively [22,50,59]. Thus, high C18:1n-9/C18:1n-7 and C20:1n-9/C18:1n-7 ratios show that plankton is the dominant dietary source, whereas low ratios indicate that sediment and suspended particulate matter has an important impact on the fatty acid composition [18,19,50,60,61].

The fundamental differences in fatty acids obtained in the present study between plankton and fishes are related to variations in ability to synthesise fatty acids between the two groups. Phytoplankton and zooplankton are known to biosynthesise fatty acids [62], such that the high levels of SFAs may be used to synthesis MUFAs and PUFAs [63]. However, fishes are unable to biosynthesise some fatty acids and therefore require them from dietary sources. The PUFAs are the essential fatty acids in fishes required for optimum growth, survival and stress resistance [64]. Accordingly, the differences in biosynthesis ability between plankton and fishes caused the observed variations in fatty acid composition. Furthermore, the fatty acids of the different fish species from the SCS were influenced by differences in dietary sources resulting from their different geographical locations, which reflect diverse sources of organic matter. Hence, analysis of specific fatty acids can reflect dietary sources in fishes.

Geographically, the nearshore SCS is close to Hainan Island, which is dominated by nutrient sources and detritus from River inputs. This area is influenced by multiple physical processes, including coastal upwelling and tidal shoaling [65,66]. The diverse physical processes regulate important environmental factors and further control the ecosystem in this area. As a result, suspended particulate matter impacted by sediments may also be an important dietary source to the nearshore fishes considering the water depth and multiple physical processes [18,60,61]. Accordingly, river inputs, coastal upwelling, and tidal-shoaling interact with each other, providing the potential for nutrients in suspended particulate matter and sediments to contribute to the diet of the nearshore fishes. Relative to the nearshore SCS, the northern continental slope of the SCS is hundreds of kilometres away from the mainland. In addition, strong bottom currents along the slope (North Pacific Intermediate Water and North Pacific Deep Water), and the Kuroshio Current from the Luzon Strait are the major factors influencing this area [34,35,67,68]. The complicated processes made the nutrient inputs of detritus from the Pearl River was hard to reach the slope area. In fact, the slope plankton contributed more to the diet of SCS slope fishes than the nearshore fishes. Moreover, the ratios of fatty acids showed that geographical factors influenced the fatty acid composition of fishes from the continental slope and nearshore areas of the SCS, through their effects on fish dietary sources.

The deep slope mesopelagic fishes were differentiated from the epipelagic fishes of the continental shelf area. The C20:1n-9/C18:1n-7 and C18:1n-9/C18:1n-7 ratios were different between the two groups of fishes. The depth of the continental shelf area was <200 m, compared with 2000–3000 m in the deep slope area. Under such conditions, upwelling could carry organic matter from the bottom to the upper water in the shallow slope area, but the potential for this to occur in the deep slope area was limited [69–71]. Compared with the epipelagic fishes, these factors have little impact on the mesopelagic fishes. The mesopelagic fishes migrated up to the epipelagic zone at night and migrated back to the mesopelagic zone during the day. The DVM and habitat of mesopelagic fishes indicated that their diet was from the euphotic layer [2], mainly plankton [6,7]. These results are consistent with the higher fatty acid signals in plankton in the mesopelagic than epipelagic fishes. In general, the mesopelagic fishes showed different fatty acid

composition than the epipelagic fishes, which is consistent with the variations in their dietary sources caused by geographical factors. Therefore, diet sources from different areas have an obvious impact on fatty acid composition of fishes.

Dietary sources and physical environmental factors affected the fatty acids composition of mesopelagic fishes

The DHA (C22:6n-3), EPA (C20:5n-3), and ARA (C20:4n-6) fatty acids have different functions in fishes. DHA plays an important role in the cell membrane [24], while EPA and ARA are precursors for eicosanoid hormones, which are involved in energy storage, immunity and reproduction [16,23,72]. Most biologically active eicosanoids are derived from ARA and EPA restrained this process. Therefore, the ARA/EPA ratio may provide an insight into the action of eicosanoids in fish physiology [16,47,72]. In most marine fishes the proportion of ARA is much lower than that of DHA and EPA, and its importance has been neglected [64]. However, the ARA/DHA and ARA/EPA ratios are essentially species-dependent but can be affected by the physical environment factors such as salinity fluctuation and temperature [73]. Therefore, the physical environment parameters are important factors affecting the fatty acid composition of fishes [16].

The high EPA/DHA ratio in the Southern Ocean plankton indicated that diatoms are the dominant phytoplankton in this area. It had previously been reported that diatoms and euphausiids were the dominant phytoplankton and macrozooplankton in the Southern Ocean, respectively [74–76]. The Southern Ocean mesopelagic fishes mainly feed on euphausiids [22]. So, they formed a simple food web. Consistent with this simple food web, the Southern Ocean mesopelagic fishes contained high signals of diatoms and had a high EPA/DHA ratio. In the SCS, diatoms made up a small proportion of the phytoplankton, as evidenced by the fatty acid signals of the plankton. Therefore, different dietary sources impacted the fatty acid composition of mesopelagic fishes.

The SCS mesopelagic fishes had higher ARA/DHA and ARA/EPA ratios compared with those in the Southern Ocean. The ARA/DHA ratio in mesopelagic fishes was not remarkably different between the two regions because of the high levels of DHA. However, the ARA/EPA ratio in SCS mesopelagic fishes was very different in fishes from the Southern Ocean. In addition, the ARA/EPA ratio in SCS mesopelagic fishes was several times higher than that in SCS plankton. The higher ARA levels might enable better adaptation to variable seawater conditions, including salinity and temperature [64]. The differences in the ARA/DHA and ARA/EPA ratios suggested that the mesopelagic fishes were impacted by physical factors in the environment. The Southern Ocean sampling stations were located near South Georgia. In this area, the salinity varies from 33.7 to 34.3, whereas the temperature ranges from 0.45°C to 8.2°C [75,77]. In the SCS the salinity ranged from 33.7 to 34.5 and the temperature varied from 4 to 27°C. The salinity varied very little in each area, and the levels were similar in both regions. Nevertheless, the SCS had a very large temperature variation from surface to middle waters, whereas in the Southern Ocean there was little temperature change. Accordingly, the SCS mesopelagic fishes needed to tolerate large temperature changes during their DVM because of the temperature variation (4°C to 27°C), and therefore needed more ARA. Thus, the temperature variation might have caused the SCS mesopelagic fishes to increase the ARA content than Southern Ocean mesopelagic fishes. Indeed, their ARA content was several times higher than that of their dietary source

(SCS plankton). Thus, the fatty acids composition of SCS mesopelagic fishes were influenced by the temperature variations in their habitat.

Conclusion

The lipid content differed between SCS epipelagic and mesopelagic fishes. When using $\delta^{13}C$ to study trophic interactions among fishes, an unusually high lipid content could cause $\Delta\delta^{13}C$ to change more than the enrichment factor in the food web. Therefore, biases must be caused by variability in lipid content when the $\delta^{13}C$ is used in the trophic interactions between mesopelagic fishes and epipelagic fishes. The extraction of lipids represented a good method for addressing the impact of lipid content in mesopelagic fishes on $\Delta\delta^{13}C$ level. In addition, the relationship between the C:N ratio and lipid content may enable the development of a mathematical normalisation method to account for the impact of lipids on $\Delta\delta^{13}C$ levels. However, different fish species have different metabolism, lipid-class composition and lifestyles, the single mathematical normalisation formula couldn't be used in all fishes. More detailed studies should be conducted in the future.

Analysis of specific fatty acid ratios indicated that both the dietary source and the physical environment (temperature) affected the fatty acid composition of SCS mesopelagic fishes. The geographical locations and diverse physical processes regulate the ecosystem in different areas. These changes will be reflected in the fatty acid composition of fishes. Furthermore, the high ARA levels enabled the mesopelagic fishes to tolerate the temperature fluctuations to which they were exposed in the SCS. The special physical environment also has an important influence on the fatty acid composition of fishes. Thus, future studies of the synthesis of fatty acids in particular species should take account of both dietary sources and physical factors in the environment.

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Appendix 1

Table A1. The lipids content, C:N ratio and $\delta^{13}C$ values of fishes in this study.

	Fish groups	
Parameter	Epipelagic fishes	Mesopelagic fishes
Lipids/%	17.0 ± 4.0^{a}	33.7 ± 2.5^{b}
C:N ratio	3.5 ± 0.3^{a}	4.6 ± 0.2^{b}
$\delta^{13}C_{bulk}$/‰	-18.8 ± 0.5^{a}	-20.1 ± 0.6^{b}
$\delta^{13}C_{extracted}$/‰	-18.4 ± 0.4^{a}	-18.8 ± 0.3^{a}

Notes: Values are mean ± SE. Mean values in the same row with dissimilar superscripts are statistically different ($p < 0.05$).