Factors affecting the fatty acid composition of mesopelagic fish of the continental slope in the South China Sea

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Study of the ecology of mesopelagic fish is central to assessing the active biological pump in the ocean, especially in the mesopelagic layer. As a consequence of the small size and pressure change, traditional stomach content analysis is not useful for analyzing the feeding behavior of mesopelagic fish. The use of δ¹³C and fatty acid analyses can help to resolve this problem. The mesopelagic fish examined in this study were collected from the continental slope in the north of the South China Sea (SCS), and are compared with nearshore SCS fish and fish collected from the Southern Ocean. The unusually high lipid content of the mesopelagic fish resulted in Δδ¹³C values (i.e., the difference in δ¹³C between unextracted and extracted tissues) exceeding 1‰, which is more than the enrichment factor in the food web. Thus, extraction of lipids was conducted prior to δ¹³C isotope analysis for the study of trophic interactions of mesopelagic fish. Compared with other fish, mesopelagic fish had high C18:1n-9/C18:1n-7 and C20:1n-9/ C18:1n-7 ratios, which confirms that plankton is their main dietary source. Diatoms comprise a higher proportion of phytoplankton in the Southern Ocean and a lower proportion in the SCS, and this is reflected in the C20:5n-3/C22:6n-3 (EPA/DHA) ratio in mesopelagic fish in each region. The low EPA/DHA ratio in SCS fish indicates that diatoms are not the main component in the diet of mesopelagic fish. The SCS mesopelagic fish had higher C20:4n-6/C22:6n-3 (ARA/DHA) and C20:4n-6/C20:5n-3 (ARA/EPA) ratios than fish in the Southern Ocean. This result suggests that physical factors (e.g., temperature) also affect the fatty acid composition of these fish, particularly because certain fatty acids enable the fish to better adapt to extreme environmental conditions. Future studies of the synthesis of fatty acids in particular species should take account of both the dietary sources and physical factors in their environment.
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Abstract
Study of the ecology of mesopelagic fish is central to assessing the active biological pump in the ocean, especially in the mesopelagic layer. As a consequence of the small size and pressure change, traditional stomach content analysis is not useful for analyzing the feeding behavior of mesopelagic fish. The use of δ^{13}C and fatty acid analyses can help to resolve this problem. The mesopelagic fish examined in this study were collected from the continental slope in the north of the South China Sea (SCS), and are compared with nearshore SCS fish and fish collected from the Southern Ocean. The unusually high lipid content of the mesopelagic fish resulted in ∆δ^{13}C values (i.e., the difference in δ^{13}C between unextracted and extracted tissues) exceeding 1‰, which is more than the enrichment factor in the food web. Thus, extraction of lipids was conducted prior to δ^{13}C isotope analysis for the study of trophic interactions of mesopelagic fish. Compared with other fish, mesopelagic fish had high C18:1n-9/C18:1n-7 and C20:1n-9/ C18:1n-7 ratios, which confirms that plankton is their main dietary source. Diatoms comprise a higher proportion of phytoplankton in the Southern Ocean and a lower proportion in the SCS, and this is reflected in the C20:5n-3/C22:6n-3 (EPA/DHA) ratio in mesopelagic fish in each region. The low EPA/DHA ratio in SCS fish indicates that diatoms are not the main component in the diet of mesopelagic fish. The SCS mesopelagic fish had higher C20:4n-6/C22:6n-3 (ARA/DHA) and C20:4n-6/C20:5n-3 (ARA/EPA) ratios than fish in the Southern Ocean. This result suggests that physical factors (e.g., temperature) also affect the fatty acid composition of these fish, particularly because certain fatty acids enable the fish to better adapt to extreme environmental conditions. Future studies of the synthesis of fatty acids in particular species should take account of both the dietary sources and physical factors in their environment.

Introduction
The rapid increase in demand for high-quality food poses great challenges for global commercial marine fisheries (Davies et al., 2009), and has stimulated the search for new fishery
resources. Mesopelagic fish, which are distributed worldwide except for the Arctic region (Catul et al., 2011), comprise a biomass of more than 10 billion tons and constitute a potential solution to this problem (Kaartvedt et al., 2012; Irigoien et al., 2014). These fish occur in the ocean at depths between 200 and 1000 m (Irigoien et al., 2014). Most mesopelagic fish migrate upward into the epipelagic layer at night and return to the mesopelagic zone in the daytime (Catul et al., 2011; Davison, 2011; Hudson et al., 2014). Because of their diel vertical migration (DVM), mesopelagic fish link primary consumers including copepods and zooplankton to higher predators including large pelagic fish, benthic fish, and marine mammals (Choy et al., 2012; Collins et al., 2012; Olivar et al., 2012). They play an important role in ocean food webs (Cherel et al., 2010; Kaartvedt et al., 2012), and their DVM behavior makes a significant contribution to the transfer of organic matter from the upper productive layer to deeper layers (Radchenko 2007; Hernández-León et al., 2010; Dypvik et al., 2012). However, the huge biomass of these fish is underutilized. Knowledge of the biological and ecological characteristics of mesopelagic fish will improve our understanding of their role in the biological pump, and enable assessment of whether they can be sustainably exploited in the future.

To address ecological questions, more research on the body composition and diet of mesopelagic fish is required. Traditional stomach content analysis does not reflect long-term feeding behavior, and readily degradable material in the diet can be underestimated (Wan et al., 2010). Fatty acid biomarker and stable isotope ($\delta^{13}$C) methods can overcome the disadvantages of stomach content analysis, and have been successfully used in studies of fish dietary sources and trophic positions (Koussoroplis et al., 2011; Cui et al., 2015). However, these methods have rarely been used in the study of mesopelagic fish.

Fatty acids and fatty acid ratios have been used as biomarkers for various food sources. Many fatty acids in the marine environment can only be biosynthesized by certain bacteria, phytoplankton, and macroalgal species, making them useful as biomarkers of these dietary sources (Stowasser et al., 2009a; Wan et al., 2010). However, extreme environmental conditions (e.g., large changes in temperature) can have marked effects on fish physiology (Arts and Kohler,
2009), as the fish must change its fatty acid composition to maintain the fluidity of their cell membranes (Parrish, 2009). Highly unsaturated fatty acids (HUFAs; FAs having ≥20 carbon atoms and ≥3 double bonds) play important structural and functional roles in adaptation to environmental stressors (Arts and Kohler, 2009). Dietary fatty acids can have profound effects on nonspecific fatty acid composition, although physical factors can also affect the content of specific fatty acids (Bell and Sargent, 2003; Koussoroplis et al., 2011).

The δ¹³C isotope signature provides a chemical record of primary production sources in higher trophic consumers (Fry, 2006; Logan and Lutcavage, 2013). The δ¹³C is more depleted in the synthesis of lipids than in the synthesis of proteins and carbohydrates (DeNiro and Epstein 1977; Pomerleau et al., 2014). Most mesopelagic fish have a higher lipid content than other fish (Stowasser et al., 2009b; Hoffman et al., 2010; Koussoroplis et al., 2011). Thus, the variation in lipid content in different fish can mask the dietary δ¹³C, making dietary reconstructions difficult (Post et al., 2007; Svensson et al., 2014). The considerable biases caused by lipid content among mesopelagic fish need to be taken into account when studying their dietary sources.

The South China Sea (SCS) is the largest semi-closed sea in the western tropical Pacific Ocean, and the second largest marginal sea worldwide (Su, 2004; Wang et al., 2011). The physical environment of the northern slope of the South China Sea (NSSCS) is complex (Su, 2004; Gong et al., 2013, 2015). Li et al. (2005) reported large diversity and numbers of mesopelagic fish on the NSSCS. However, there is limited knowledge of the ecology of mesopelagic species in this region. A greater understanding of their body composition and diet would help clarify the role of mesopelagic species in the active biological pump, and enable assessment of their potential for sustainable exploitation in the future.

In this study, we investigated the ecological characteristics of mesopelagic fish from the NSSCS using fatty acid and stable isotope analyses. The factors potentially controlling the composition of fatty acids were also evaluated by comparison of samples from the near-shore region of the SCS and the Southern Ocean. In particular, we studied the impact of lipid content on the δ¹³C of mesopelagic fish, as this is essential for further studies of trophic interactions.
2. Materials and methods

2.1 Sampling

Fish were collected from the continental slope of the SCS during a cruise carried out in October 2014 (R/V Nan Feng), and sampling of the nearshore SCS was carried out in May 2011 using a local fishing boat (Fig. 1). The fish from the shallow slope of the SCS (stations L1 and L4) were caught using a bottom trawl having a 150 m mouth perimeter and a 51.5 m headope length. Fish from the deep slope of the SCS (stations L2 and L3) were caught using a mid-layer trawl having a 136.1 m mouth perimeter and a 30.0 m headope length. Plankton were collected by vertical trawling using a net having mesh sizes of 76, 167, and 505 µm. The samples were washed using filtered seawater, and filtered onto pre-combusted (450°C, 5 h) 47 mm GF/F filters. All samples were stored frozen at –20°C until transferred to the laboratory. In the laboratory, muscle tissue was excised from below the dorsal fin, and the skin and scales were removed. The tissue samples were lyophilized in a freeze dryer (LOC-1; Christ, Germany) and stored at –40°C until analysis (Cui et al., 2012, 2015). The dried muscle was powdered using a mortar and pestle.

Sampling dates, locations, and water depths are listed in Table 1. Data on mesopelagic fish and plankton from the Southern Ocean were obtained from previous publications (Stubing and Hagen, 2003; Stowasser et al., 2009b).

2.2 Fatty acid analysis

The fatty acid composition was determined from a known quantity of tissue extracted using a dichloromethane–methanol solvent system (2:1 v/v, using 0.01% BHT), based on the Folch method for total lipid determination (Folch et al., 1957; Cui et al., 2015). For the dorsal muscle samples, approximately 15 ml of a mixture of dichloromethane and methanol (2:1) was added to 100 mg of sample. The mixture was extracted and centrifuged (3000 rpm, 10 min), and the upper organic solvent layer was transferred to a flask using a pipette. The solvent was evaporated to dryness under a stream of N₂ at room temperature, weighed, and the lipid content was calculated as weight percent of the unextracted freeze dried tissue (Svensson et al., 2014). The extracted
tissue was dried under a stream of N\textsubscript{2} at room temperature and used for \(\delta^{13}\)C measurement.

The fatty acids were transformed to fatty acid methyl esters (FAMEs) using a mixture of methanol (containing 5% HCl) and n-hexane, and held at 50\degree C for approximately 12 h (Eder, 1995). The FAMEs were analyzed using gas chromatograph mass spectrometry (7890A GC with a 5975C MSD; Agilent, USA) equipped with a DB-FFAP capillary column (30 m length, 0.25 mm i.d., 0.25 \(\mu\)m film thickness; Agilent, USA). As an internal recovery standard, C21:0 was added to the samples, and C19:0 methyl ester was added as an internal quantification standard. The injector and detector temperatures were both 250\degree C. Injections (1 \(\mu\)l) were made at 60\degree C, and the temperature was increased to 170\degree C at a rate of 30\degree C/min. The temperature was held constant for 5 min, then increased to 220\degree C at 1\degree 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\textsuperscript{TM}). The content of particular fatty acids is expressed as the relative percentage of the total fatty acid content, based on peak areas. The fatty acid recovery rate in the analysis was >80\%. If more than one sample was analyzed, the data are reported as the mean \pm the standard deviation (SD). Fatty acids are named using the shorthand notation CA:Bn-X, where A indicates the number of carbon atoms, B is the number of double bonds, and X indicates the position of the first double bond relative to the terminal methyl group (Budge et al., 2006; El-Sabaawi et al., 2010).

2.3 Stable isotope analysis

Stable carbon isotope ratios (\(\delta^{13}\)C) were measured for tissue before extraction (unextracted tissue; \(\delta^{13}\)C\textsubscript{bulk}) and following extraction (extracted tissue; \(\delta^{13}\)C\textsubscript{extracted}). Dried powdered samples were weighed into tin cups for \textsuperscript{13}C analysis. Stable carbon and nitrogen isotopes were measured using an isotope ratio mass spectrometer (Finnegan Delt plus XP; Thermo, Germany). The results were normalized to Vienna Pee Dee Belemnite standard (PDB) for \(\delta^{13}\)C (Overman and Parrish, 2001). The stable isotope ratios are expressed in \(\delta\) notation of units per mill as follows (Pomerleau et al., 2014; Cui et al., 2015):
\[ \delta X (\%) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \]

where \( X = ^{13}\text{C} \) and \( R = ^{13}\text{C}/^{12}\text{C} \). The precision of the stable isotope analyses was \( \pm 0.1\% \). The C:N ratio is expressed as a molar ratio.

2.4 Statistical analysis

For \( ^{13}\text{C} \) values, paired comparisons were made between extracted tissue (\( ^{13}\text{C}_{\text{extracted}} \)) and unextracted tissue (\( ^{13}\text{C}_{\text{bulk}} \)). All data on fatty acid compositions are presented as the mean \( \pm \) SD.

Principal component analysis (PCA) was used to investigate variation in the fatty acid signatures among fish species, and to identify which fatty acids were most responsible for the variation (Stowasser et al., 2009b). PRIMER 5.0 and SPSS 23.0 software were used for data analysis (Prigge et al., 2012). The data were transformed (arcsine of the square root) to ensure normality (percentage data), or log 10 transformed to ensure homogeneity of variances (Li et al., 2011).

Based on the PCA scores the fish were separated into several groups based on species and locations. Similarity analysis was performed to determine whether the differences in fatty acid composition among the groups were significant.

3. Results

3.1 Fish lipid content, C: N, and \( \Delta^{13}\text{C} \)

The fish lipid content, C:N, and \( \Delta^{13}\text{C} \) values (i.e., the difference in \( ^{13}\text{C} \) value between unextracted and extracted tissue) are listed in Table 2. Fish from the shallow slope of the SCS were considered SCS epipelagic fish, based on their water layer habitat. Fish from the deep slope of the SCS were considered SCS mesopelagic fish because of their DVM behavior in the deep slope of the SCS. Analysis of the lipid content, C:N, and \( \Delta^{13}\text{C} \) parameters showed large differences between epipelagic and mesopelagic fish. The mesopelagic fish had a higher lipid content, C:N, and \( \Delta^{13}\text{C} \) than the epipelagic fish. There was a significant effect of lipid extraction on the \( ^{13}\text{C} \) values for the mesopelagic fish. Following lipid extraction, the \( ^{13}\text{C} \) value for the mesopelagic fish changed by \( >1\% \), while for epipelagic fish the change was less than precision, and for \textit{Nemipterus bathybius} and \textit{Decapterus macrosoma} there was almost no change (Table 2).
Figure 2 shows significant positive relationships between the lipid content and the C:N ratio ($P < 0.001$, $R^2 = 0.83$), the C:N ratio and $\Delta^{13}C$ ($P < 0.001$, $R^2 = 0.64$), and the lipid content and $\Delta^{13}C$ ($P < 0.001$, $R^2 = 0.73$).

### 3.2 Fatty acid composition of SCS fish

The fish from the SCS varied in their fatty acid compositions. For most fish, polyunsaturated fatty acids (PUFA) were the major compounds, accounting for 30%–60% of the total fatty acid content. The saturated fatty acid (SFA) content did not vary substantially amongst all fish. 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 acid content, with other fatty acids typically comprising <3%.

The PCA results show good separation of the fatty acid signatures of the fish and plankton. The PCA identified four main groups (Fig. 3a), with components PC1 and PC2 accounting for 55.4% of the variation. The plankton and fish were separated into two distinct groups. Based on factor loading plots (not shown), 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 fish. The content of C14:0 in plankton was about 7%–15%, compared with 0.7%–4% in fish. In addition, the C18:4n-3, C15:0, and C18:3n-3 contents in plankton were >1%, compared with <1% in fish; the fish had more C22:6n-3 than did the plankton. Comparison of fish from different areas shows that the SCS slope fish had higher percentages of C18:1n-9 and lower percentages of C18:1n-7 than the nearshore SCS fish. The SCS mesopelagic fish had the highest C18:1n-9/C18:1n-7 and C20:1n-9/C18:1n-7 ratios, with the nearshore fish having the lowest ratios and the SCS epipelagic fish having intermediate values (Fig. 4b). Based on differences in the ratios of specific fatty acids, the mesopelagic fish could be separated from the epipelagic fish using PCA.

### 3.3 Fatty acid composition of mesopelagic fish

The SCS mesopelagic fish had stable SFA contents. PUFAs were the major compounds, accounting for 30%–54% of the total fatty acid 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 fatty acid
content. Other fatty acids typically comprised <3%. However, Southern Ocean mesopelagic fish have been reported to show variable contents of various fatty acids (Stowasser et al., 2009b).

Based on the PCA, the mesopelagic fish and plankton from the SCS and the Southern Ocean were separated into four groups based on fatty acid contents (Fig. 5a). The four groups include the SCS plankton, the Southern Ocean plankton, the SCS mesopelagic fish, and the Southern Ocean mesopelagic fish. PC1 separated the groups into different oceanic regions, while PC2 separated them into plankton and mesopelagic fish. Based on factor loading plots (not show) the C22:6n-3, C16:0, C18:1n-7, C20:4n-6 and C20:1n-9 fatty acids were shown to have 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 result. The plankton and mesopelagic fish 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 >1. The plankton and mesopelagic fish from the SCS had higher C20:4n-6/C22:6n-3 (ARA/DHA) and C20:4n-6/C20:5n-3 (ARA/EPA) ratios than those from the Southern Ocean. The ARA/EPA ratio in SCS mesopelagic fish was several times higher than that in the SCS plankton (Fig. 5b).

4. Discussion

4.1 Impact of lipid content on fish

In the traditional method δ¹³C is moderately enriched (<1‰) during trophic transfer (Layman et al., 2012; Pomerleau et al., 2014). The δ¹³C analysis provides information regarding the sources of primary production in the ecosystem, and the flow of carbon from primary producers to consumers (Logan and Lutcavage, 2013). However, carbon discrimination occurs during lipid synthesis, and as a result the δ¹³C of lipids is more depleted than that of proteins and carbohydrates (Hoffman and Sutton, 2010). Furthermore, a high lipid content can mask the origin of the carbon incorporated into the tissues of an organism (DeNiro and Epstein 1977; Pomerleau et al., 2014). Therefore, the lipid content can strongly influence the interpretation of δ¹³C data.

In the present study, the lipid content of mesopelagic fish was >30% (Table 2), similar to the
content reported for mesopelagic fish of the Southern Ocean, but higher than that reported for freshwater and coastal fish (Stowasser et al., 2009a; Hoffman et al., 2010). A high lipid content enables mesopelagic fish to easily adapt to daily vertical migrations (Catul et al., 2011; Davison, 2011; Hudson et al., 2014). These vertically migrating fish have high levels of lipids for activity and energy storage (Childress et al., 1980). Thus, the high lipid content plays an important role in the migration of mesopelagic fish and is a unique characteristic of such fish. In the present study, the $\Delta \delta^{13}C$ value for lipids was usually $>1\%$, which exceeds the typical level of $\delta^{13}C$ enrichment in the food chain (Fig. 2a). Comparison of trophic levels based on $\delta^{13}C$ values shows that carbon discrimination leads to the differences between mesopelagic fish and species having a low lipid content, including epipelagic fish. 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 of the trophic structure of mesopelagic species.

The C:N ratio was positively correlated with the lipid content; its value was controlled by the molecular structure of protein and lipid (Post et al., 2007; Svensson et al., 2014). The C:N ratio may reflect the lipid content to some extent. The precision of the $\delta^{13}C$ analysis was $\pm 0.1\%$. In our study the impact of lipids could be ignored when the lipid content of fish tissues was $<17\%$ (C:N $< 3.5$), which is consistent with the reports of Post et al. (2007) and Layman et al. (2012). However, in our study the mesopelagic fish had a lipid content $>30\%$ (C:N $> 4.4$), and this could have a substantial impact on analyses. If established methods are used to study the dietary sources of mesopelagic fish, erroneous results are inevitable. Consequently, lipid correction is particularly important in analyses of mesopelagic fish. The relationship between the C:N ratio and the lipid content could be used to develop a mathematical normalization method in future studies to overcome the impact of lipid content.

4.2 Geographical location affected the fatty acid composition of SCS fish

Fatty acids and fatty acid ratios are used as biomarkers of different food sources, and their analysis 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 (Dalsgaard et al., 2003; Kattner and Hagen, 2008; Stowasser et al., 2009b). Thus, high C18:1n-9/C18:1n-7 and C20:1n-9/C18:1n-7 ratios show that plankton is the dominate dietary source, whereas low ratios indicate that organic matter was obtained mainly from sediment and suspended particulate matter.

Figure 3a shows that the PCA analysis separated the plankton and fish from the continental slope and nearshore SCS into four groups. In this context, there are fundamental differences between plankton and fish. Phytoplankton and zooplankton can biosynthesize fatty acids (Hagen and Auel, 2001), but fish are unable to biosynthesize some fatty acids; they acquire these from dietary sources. Because of physiological differences, the plankton and fish show differences in fatty acid contents. Furthermore, the fatty acids of the fish from the SCS and the Southern Ocean 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 because the source impacts the fatty acid compositions of fish.

Geographically, the nearshore SCS is close to Hainan Island. River inputs of detritus from Hainan Island are the dominant nutrient source to this ecosystem, which is influenced by multiple physical processes, including coastal upwelling and tidal shoaling (Liu et al., 2011; Song et al., 2012). These phenomena lead to bathymetric variations in ecosystem characteristics, and diverse physical processes regulate important environmental factors and further control the ecosystem in this area. In view of the water depth and the multiple physical processes, organic matter from sediments may also be an important dietary source for nearshore fish. 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 nearshore fish. Relative to the nearshore SCS, the northern continental slope of the SCS is hundreds of kilometers 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 major factors influencing this area (Yang et al., 2010; Gong et al., 2013, 2015; He et al., 2013). The
complicated processes made the nutrient inputs of detritus from the Pearl River was limited. Compared with the nearshore fish, the slope plankton contributed more to the diet of SCS slope fish. The ratios of characteristic fatty acids show that geographical factors influenced the fatty acid compositions of fish from the continental slope and nearshore areas of the SCS, through their effects on fish dietary sources.

Figure 4a shows that the deep slope mesopelagic fish were differentiated from the epipelagic fish of the shallow slope area. The C20:1n-9/C18:1n-7 and C18:1n-9/C18:1n-7 ratios are different between the two groups of fish. The depth of the shallow slope area is <200 m, compared with 2000–3000 m in the deep slope area. Shallow slope upwelling could carry organic matter from the sediment to the upper water in the shallow slope area, but the potential for this to occur in the deep slope area is limited. Compared with the epipelagic fish, these factors have little impact on the mesopelagic fish. The mesopelagic fish migrate up to the epipelagic zone in the night, and migrated back to the mesopelagic zone during the day. The diel vertical migration and habitat of mesopelagic fish indicate that their diet is from the euphotic layer (Catul et al., 2011), mainly plankton in this layer (Davison, 2011; Hudson et al., 2014). The present results are consistent with this view, as the fatty acid signals of plankton were higher in mesopelagic than epipelagic fish. Briefly, the mesopelagic fish showed a different fatty acid composition than the epipelagic fish, which is consistent with differences in their dietary sources caused by geographical factors.

4.3 Dietary sources and physical environment affect the fatty acid composition of mesopelagic fish

The DHA (C22:6n-3), EPA (C20:5n-3), and ARA (C20:4n-6) fatty acids have different functions in fish. DHA plays an important role in the cell membrane (Arts and Kohler, 2009), while EPA and ARA are precursors for eicosanoid hormones, which are involved in energy storage, immunity, and reproduction (Schmitz and Ecker, 2008; Parrish, 2009; Koussoroplis et al., 2011). More biologically active eicosanoids are derived from ARA than from EPA, although EPA restrained this process. Therefore, the ARA/EPA ratio may mirror the action of eicosanoids.
in fish physiology (Sargent et al., 1999; Schmitz and Ecker, 2008; Koussoroplis et al., 2011). In most marine fish the proportion of ARA is much lower than that of DHA and EPA, and its importance has been neglected (Bell and Sargent, 2003). However, the ARA/DHA and ARA/EPA ratios are essentially species-dependent, but can be affected by the environment (Recks and Seaborn, 2007). Therefore, the physical environment is an important factor affecting the fatty acid composition of fish (Koussoroplis et al., 2011).

As described above, plankton and mesopelagic fish use different fatty acids for physiological activities. The plankton synthesize fatty acids, but the fish assimilate essential fatty acids from their dietary sources. Therefore, the plankton and mesopelagic fish show large differences in their fatty acid compositions.

The Southern Ocean plankton have a high EPA/DHA ratio (> 1), indicating that diatoms are the dominant phytoplankton in this area. It has previously been reported that diatoms and euphausiids are the dominate phytoplankton and macrozooplankton in the Southern Ocean, respectively (Schmidt et al., 2012; Ward et al., 2012a, 2012b). The Southern Ocean mesopelagic fish mainly feed on euphausiids (Stowasser et al., 2009b) in a simple food chain comprising diatoms, euphausiids, and mesopelagic fish. Consistent with this simple food chain, the Southern Ocean mesopelagic fish contained high signals of diatoms and had a high EPA/DHA ratio. In the present study area, diatoms made up a small proportion of the phytoplankton, and this is mirrored in the fatty acid signals of the plankton. Therefore, different dietary sources impacted the fatty acid compositions of mesopelagic fish.

Compared with those in the Southern Ocean, the SCS mesopelagic fish had higher ARA/DHA and ARA/EPA ratios. Because of the high levels of DHA, the ARA/DHA ratio in mesopelagic fish was not remarkably different between the two regions. However, the ARA/EPA ratio in SCS mesopelagic fish was very different from that in fish from the Southern Ocean. In addition, the ARA/EPA ratio in SCS mesopelagic fish 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 (Bell and Sargent, 2003). The differences in the ARA/DHA and
ARA/EPA ratios suggest that the mesopelagic fish 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, and the temperature ranges from 0.45°C to 8.2°C (Young et al., 2011; Ward et al., 2012a). In the SCS the salinity ranged from 33.7 to 34.5 and the temperature range was 2–27°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 bottom waters, whereas in the Southern Ocean there was little temperature change. Because of the temperature variation (4°C to 27°C), during their diel vertical migration, SCS mesopelagic fish need to be able to tolerate large temperature changes, and therefore need more ARA. Thus, the temperature variation leads to SCS mesopelagic fish having a greater ARA content than Southern Ocean mesopelagic fish. Moreover, their ARA content is several times higher than that of their dietary source (SCS plankton). Thus, the fatty acid composition of SCS mesopelagic fish is influenced by both their dietary source and temperature variations in their habitat.

Conclusion

The lipid content differs between SCS epipelagic and mesopelagic fish. When using δ¹³C to study trophic interactions among fish, an unusually high lipid content can cause Δδ¹³C to change more than the enrichment factor in the food web. Therefore, biases caused by variability in lipid content must be normalized. The extraction of lipids represents a good method for addressing the impact of lipid content in mesopelagic fish on Δδ¹³C levels. In addition, the relationship between the C:N ratio and lipid content may enable the development of a mathematical normalization method to account for the impact of lipids on Δδ¹³C levels.

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 fish. Compared with the nearshore fish and SCS epipelagic fish, plankton were the dominate dietary source of SCS mesopelagic fish, as their geographical location meant that they sourced little
organic matter from detritus and sediment. Therefore, dietary sources probably affect the fatty acid composition of SCS mesopelagic fish, which have higher ARA/DHA and ARA/EPA ratios than fish from the Southern Ocean. Furthermore, the ARA/EPA ratio in mesopelagic SCS fish was several times higher than that in SCS plankton. The high ARA levels enable the mesopelagic fish to tolerate the temperature fluctuations to which they are exposed in the SCS. The physical environment also has an important influence on the fatty acid composition of fish. Future studies of the synthesis of fatty acids in particular species should take account of both dietary sources and physical factors in the environment.

Acknowledgements

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Topical Studies in Oceanography 58, 1540-1552.
Table 1 (on next page)

Data related to fish in this study.
Table 1. Data related to fish in this study

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<tr>
<th>Station</th>
<th>Longitude</th>
<th>Latitude</th>
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<th>Time</th>
<th>Bottom Depth (m)</th>
<th>Sample Depth (m)</th>
<th>Species (N)</th>
<th>Length (mm)</th>
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<td>120</td>
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Table 2 (on next page)

Lipid, C:N, and \(^{13}\)C values of fish in this study.
<table>
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<tr>
<th>Species</th>
<th>Lipid (%)</th>
<th>C:N</th>
<th>$\delta^{13}$C (%)</th>
<th>$\Delta\delta^{13}$C (%)</th>
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<td>Unextracted</td>
<td>Extracted</td>
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<td>-18.3</td>
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<td>-18.7</td>
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<td>-19.0</td>
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<td>-18.7</td>
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<td>4.1</td>
<td>-19.6</td>
<td>-18.8</td>
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<td>Mesopelagic fish</td>
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<td>-19.4</td>
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<td>4.8</td>
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</table>
Figure 1

Locations of sampling stations in the South China Sea.
Figure 2

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 $\Delta \delta^{13}C$. 
Figure 3

Principal component analysis of fatty acids in SCS plankton and fish.

![Principal component analysis of fatty acids in SCS plankton and fish.](image)
Figure 4

Principal component analysis of fatty acids in SCS slope fish.

[Graph showing principal component analysis with axes PC1 (36.3%) and PC2 (17.2%). Red triangles represent SCS epipelagic fish, and cyan squares represent SCS mesopelagic fish.]
Figure 5

Ratios of characteristic fatty acids in SCS fish.
Figure 6

Principal component analysis of fatty acids in plankton and mesopelagic fish.

Data on mesopelagic fish and plankton from the Southern Ocean were obtained fromStubing and Hagen (2003) and Stowasser et al., (2009b)
Figure 7

Ratios of characteristic fatty acids in plankton and mesopelagic fish.