Chinese Journal of Oceanology and Limnology
Vol. 34 No. 5, P. 1085-1096, 2016
http://dx.doi.org/10.1007/s00343-016-4309-9

Compound-specific isotopes of fatty acids as indicators of trophic interactions in the East China Sea ecosystem*

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Received Mar. 14, 2015; accepted in principle Jun. 15, 2015; accepted for publication Jul. 22, 2015

Abstract The composition and compound-specific isotopes of fatty acids were studied within food webs in the East China Sea. Lipid-normalized stable carbon isotopes of total organic carbon had a good correlation with trophic level. Variations in fatty acid compositions among different species were observed but were unclear. Different dietary structures could be traced from molecular isotopes of selected fatty acids in the Shiba shrimp (*Matapenaeus joyneri*), the coastal mud shrimp (*Solenocera crassicornis*) and the northern Maoxia shrimp (*Acetes chinensis*). Both *M. joyneri* and *S. crassicornis* are mainly benthos feeders, while *A. chinensis* is a pelagic species, although they have a similar fatty acid composition. There was a good correlation for isotopes of arachidonic acid (C20:4n6; ARA) and docosahexaenoic acid (C22:6n3; DHA) among pelagic species from higher trophic levels. The isotopic compositions of DHA in benthic species were more negative than those of pelagic species at the same trophic level. The fact that the diet of benthic species contains more degraded items, the carbon isotopes of which are derived from a large biochemical fraction, may be the reason for this variation. A comparative study of benthic and pelagic species demonstrated the different carbon sources in potential food items and the presence of a more complex system at the water-sediment interface.

Keyword: fatty acid; compound-specific isotope ratio; stable isotope ratio; East China Sea

1 INTRODUCTION

In China, the East China Sea is the location of important marine fishing grounds. The northern coastal zone is particularly important because it is the spawning and feeding ground of many fish species. For over the last half century, anthropogenic disturbance (overfishing and pollution) and global climate change have resulted in changes in the structure of the fish community. Both landings and biodiversity have decreased dramatically, and some dominant species (e.g., the large yellow croaker (*Larimichthys crocea*)) have vanished completely (Cheng et al., 2006). Currently, the dominant species in the East China Sea are little yellow croaker (*Pseudosciaena polyactis*) and hairtail (*Trichiurus haumela*), but their stocks too are declining (Li et al., 2004). Trophic interactions within the food web in the East China Sea are inadequately studied. Thus, more information would improve our understanding of the diversity and vulnerability of this marine ecosystem under conditions of changing climate and high anthropogenic disturbance (Tang, 1999; Cai et al., 2005; Kim et al., 2005; Zhang et al., 2007).

Traditionally, gut content analysis or weighted average formulas have been used to elucidate food-web dynamics and community structure (Jin et al., 2003; Hughes et al., 2004; Alfaro, 2008). Gut content analyses require large sample sizes and are time-consuming but relatively easy and inexpensive to conduct. However, they only provide information on

* Supported by the National Basic Research Program of China (973 Program) (Nos. 2011CB409801, 2014CB441502)
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ingested food and not the type of assimilated food types, while the different rates of digestion among prey species may seriously bias estimates. Moreover, the gathered information only provides a snapshot of the most recent meal and may not be representative of the longer-term diet (Iverson et al., 2004). Nevertheless, in light of such strengths and limitations, gut content analysis still plays an important role in the study of diet, especially for larger species (Alfaro, 2008).

Because the composition of stable isotopes in tissue is correlated with diet, trophic level and body condition, their analysis can evaluate the relative importance of dietary components to a consumer (Vander Zander and Rasmussen, 2001; Post, 2002; Ruess et al., 2005; Jaeger et al., 2010). Vander Zander and Rasmussen (2001) reported that stable isotope techniques can provide a time-integrated measure of trophic position, including trophic omnivory, and that they can be used to track energy or mass flow through ecological communities. However, isotopic routing, wherein isotopes are incorporated differentially into various tissues and compounds, is a major limitation of this approach (Petersons and Fry, 1987; Gannes et al., 1997).

Fatty acids are ubiquitous in marine organisms, which contain high levels of long-chain, polyunsaturated fatty acids that originate from various unicellular phytoplankton and seaweeds (Dahl et al., 2003; Alfaro et al., 2006). From the perspective of animal nutrition, fatty acids may be grouped into essential fatty acids (EFAs), which cannot be synthesized de novo but are essential to growth and development, and those that can be synthesized (nonessential). The EFAs are mainly from the linoleic acid family (such as 18:2n6 and 20:4n6) and linolenic acid family (such as 18:3n3, 20:5n3 and 22:6n3) (Leaf, 1993). For marine species, 20:4n6, 20:5n3 and 22:6n3 are considered the three most important EFAs. They may affect many cellular and physiological processes, including cold adaptation and survival, which are indispensable for finfish growth and stress resistance (Bell and Sargent, 2003). Biochemical restrictions on the synthesis of fatty acids in many predators mean that they cannot synthesize them themselves (i.e., they must come from a food source (EFA)); therefore, fatty acids are valuable biomarkers of various food sources (such as different types of prey). Furthermore, some fatty acids are transferred from primary producers to higher trophic levels without structural changes (Saito et al., 2002). Thus, these biomarkers can be used to trace the origin and transfer of organic matter within marine ecosystems (Auel et al., 2002; Graeve et al., 2002; Iverson et al., 2002, 2004; Choy et al., 2008). Fatty acid profiles may provide useful dietary information (especially assimilated food data) for groups with known and unequivocal lipid biomarkers, but they are not available for many taxa (Alfaro, 2008). As reported in previous studies, 20:5n3 and the 20:5n3/22:6n3 ratio are the indicators of phytoplankton, 20:1+22:1 are indicators of zooplankton and macroalgae (18:1n9/20:5n3/20:4n6) and 18:1n7 and $\sum$15+$\sum$17 are indicators of bacterial input (Alfaro, 2008).

When dietary fatty acids are routed into predator tissues without degradation, their isotopic composition is similar to that of the corresponding fatty acid in the food source (Ruess et al., 2005). Moreover, fatty acids synthesized de novo in the predator can be used to trace the isotopic values of carbon catabolized from dietary macronutrients (Ruess et al., 2005). Studies have shown that compound-specific isotope compositions provide useful information about the sources of organic matter and environmental changes (Goñi and Eglinton, 1996; Countway et al., 2007), and have been applied in food-web research (Fantle et al., 1999; Müller-Navarra et al., 2000; Budge et al., 2008). If isotopically distinct dietary sources exist, it is possible to determine the relative contribution of different dietary items to a consumer (Phillips and Gregg, 2003; Phillips et al., 2005; Budge et al., 2008). In Arctic ecosystems, because of the distinctive isotopic composition of the short-chain fatty acids of phytoplankton and ice algae, quantitative estimations of the contribution of ice algae and phytoplankton to higher trophic biota have been successfully applied (Budge et al., 2008).

In the present study, compound-specific isotopes of fatty acids were used to identify the trophic interactions among dominant producers and consumers in the East China Sea. Because dietary preferences can differ with sex and body size, the corresponding shifts in compound-specific isotopes were compared and studied. A detailed comparison between benthic and pelagic species collected from the East China Sea was also conducted to elucidate differences in dietary composition and trophic level.

2 MATERIAL AND METHOD

2.1 Sample collection

The majority of samples were collected during a

Table 1 Sample data

Species	Tissue type	Sample type	Collection date	Water depth (m)	Description	Trophic level*
Calanus sinicus	Whole body	Mixture from one station	May, 2007		Copepods, zooplankton	2.0
Sagitta robusta	Whole body	Mixture from one station	May, 2007		Arrow worm, zooplankton	2.0–2.3
Acetes chinensis	Whole body	Mixture from one station	May, 2007		Shrimp, zooplanktivores	2.1
Metapenaeus joyneri Miers	Whole body	Mixture from one station	May, 2007	38	Shrimp, benthivores	2.2
Solenocera crassiocornis	Whole body	Mixture from one station	May, 2007	47	Shrimp, benthivores	2.2
Thrissa kammalensis	Dorsal muscle	Individual sample from one station	May, 2007	44	Fish, zooplanktivores	3.2
Trachurus japonicus	Dorsal muscle	Individual sample from one station	May, 2007	75	Fish, piscivores	3.4
Harpodon nehereus	Dorsal muscle	Individual sample from one station	May, 2007	44	Fish, piscivores	3.5
Pseudosciaena polyactis	Dorsal muscle	Mixture of different lengths from one station	May, 2007	15	Fish, shrimp/ fish predators	2.7–3.6
Trichiurus haumela	Dorsal muscle	Individual sample from one station	May, 2007	62	Fish, piscivores	3.9–4.1

* Wei and Jiang, 1992; Zhang and Tang, 2004; Cai et al., 2005.

survey conducted in May 2007 in the northern East China Sea (120°–125°E, 27°–33°N) by the R/V *Beidou*. Zooplankton were sampled by vertical tows from 30 m depth to the sea surface using 500-μm mesh nets. Samples were concentrated by filtration through a 20-μm mesh and then separated manually into general classes (copepods (*Calanus sinicus*) and arrow worms (*Sagitta robusta*)). These were then collected on a GF/F filter and stored at -20°C.

To collect fish species, bottom trawls were conducted using an 836-mesh, 12-cm circumference net with a 10-cm mesh cod-end and 2.4-cm mesh liner. The duration of the trawl hauls varied between 0.5 and 1.5 h at a hauling speed of ~3 kt. Water depth at the sampling stations is illustrated in Table 1. All samples were stored at -20°C until analyzed in the laboratory.

Detailed information on selected samples is listed also in Table 1. Owing to sampling difficulties, limited samples were only available for some species, and adult individuals of these species were selected. For lower trophic level samples, such as copepods, arrow worms and the shrimp *Acetes chinensis*, mixtures of tissue from various samples from the same station were used for further analysis. Trophic-level data were derived from previous studies in the same area (Wei and Jiang, 1992; Zhang and Tang, 2004).

2.2 Analysis of stable isotopes

Detailed data of pretreatment are available in the literature (Wan et al., 2010). Briefly, fish samples were dissected using stainless steel scalpels, and the gut contents were removed. All samples, including zooplankton, shrimps, and fish muscle tissue, were freeze-dried, homogenized and ground to a fine powder with an agate mortar and pestle (Table 1). Because the various biochemical components differ isotopically, stable isotope analysis of food webs must consider the strong discrimination against ^{13}C (i.e., more negative $\delta^{13}C$ values) in lipid-rich tissues. Therefore, a lipid-normalizing model for different aquatic biota was established and applied to the East China Sea ecosystem (Wang et al., 2009).

Stable carbon isotopes were measured using a Finnigan Delta$^+$ XP IR-MS system. Isotope ratios were expressed as conventional delta (δ) values in parts per thousand (or per mil, ‰). Differences between the isotope ratio of a sample and that of an international standard were calculated according to the following formula:

$$\delta^{13}C=[(R_{sample}/R_{standard})-1]\times10^3,$$

$$R=^{13}C/^{12}. \quad (1)$$

The $\delta^{13}C$ of a pure carbon dioxide reference gas was measured as the criterion for carbon, and standard deviations of the isotopic measurements were <0.1‰.

2.3 Lipid extraction and analysis

A modified Folch method (Folch et al., 1957) was used to extract lipids from all samples. The extracts were transferred to vials and reduced under a stream of nitrogen. Fatty acids were obtained via saponification followed by re-extraction into hexane. Esters of the fatty acids were prepared by

transesterification with 14% BF_3-MeOH for 1 h at 80°C. The analyses were performed using a Varian 3800 gas chromatograph equipped with a flame ionization detector (FID), an SP 2380 column (30 m× 0.25 mm i.d.) and a split injector system. Nitrogen was used as the carrier gas (Alfaro et al., 2006) and the FID detector was kept at 260°C during analysis. The following temperature program was used: 50°C for 2 min, ramped to 200°C at 4°C/min, and held at 250°C for 20 min after ramping at 10°C/min. Individual components were identified by comparing retention-time data with a standard (Supelco 37 component FAME Mix, No. 47885). Recoveries for the whole analysis procedure for fatty acids were in the range 87%–112%, calculated by comparison with that of an internal standard C19:0 fatty acid methyl ester of known concentration. Averaged data for the individual fatty acids are expressed as mass percentages of total fatty acids.

2.4 Compound-specific stable C isotope analysis of fatty acids

Gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) analyses were conducted using a Thermo Finnigan Deltaplus XP system. The GC was fitted with a fused-silica capillary column (DB-5ms, 50 m×0.32 mm i.d., 0.25-μm film thickness). Although the stationary phase of the DB-5 column has a lower separation capability for isomers than an SP-2380 column, the increased length of the DB-5ms column and lower bleeding could make up for the difference and, thereby, yield satisfactory results (Meier-Augenstein, 2002). The temperature of the oven was held at 50°C for 2 min, increased to 200°C at a rate of 10°C/min, increased to 240°C at 3°C/min and held for 5 min, and then increased to 280°C at 2°C/min and held for 10 min. Helium was used as the carrier gas. Internal standards (19:0 FAME) of known $\delta^{13}C$ value were measured every sixth run to monitor any fluctuations in instrumental measurements over time. The standard deviation of the IR-MS analyses was about 0.5‰ based on internal standard measurements (n=11). The precision of triplicate GC/C/IRMS analyses conducted for each sample was <1‰. Fatty acid $\delta^{13}C$ values were corrected for the carbon added during methylation as shown below, because there was no evidence of a kinetic isotope effect associated with transesterification (Ballentine et al., 1996):

$$\delta^{13}C_{FAME}=f_{FA}\times{}^{13}C_{FA}+f_{Methonal}\times{}^{13}C_{Methonal}. \quad (2)$$

2.5 Statistical analysis

Data were analyzed using ANOVA. Levene's test was used for homogeneity of variance. Means were compared by Tukey's HSD test. Statistical analyses were performed using SPSS 17.0 for Windows (Apache Software Foundation, Forest Hill, MD, USA).

3 RESULT

3.1 Fatty acid compositions

Table 2 lists selected relative fatty acid compositions of various species with carbon chain lengths ranging from 15 to 22. About 40 compounds were detected and identified. For most species, 16:0 (palmitic acid), 16:1n7, 18:0, 18:1n9, 20:4n6 (arachidonic acid (ARA)), 20:5n3 (eicosapentaenoic acid (EPA)), and 22:6n3 (docosahexaenoic acid (DHA)) dominated, but their levels varied markedly among species. Higher 20:1n9 (>3%) and 22:1n11 levels were found in zooplankton samples (copepods and arrow worms) and in zooplanktivores (*A. chinensis* and *Thryssa kammalensis*). Markedly high levels of 20:4n6 (6%–8%) were observed in benthivores.

Lower percentages of 20:5n3 and 22:6n3 were found in the fish species *T. kammalensis* and *Trachurus japonicus* than reported in a previous study (Wan et al., 2010). Higher levels of saturated fatty acids (SFAs) were observed in these fish samples than in zooplankton. A lower percentage of SFAs and a higher percentage of polyunsaturated fatty acids (PUFAs) were found in the fish species *Harpodon nehereus*, *P. polyactis* and *T. haumela* compared with other species. For the various species, the highest percentages of n6 PUFAs were detected in benthic species, such as *Solenocera crassicornis*. Generally, the n6 PUFA of fish samples represented <6% of the total fatty acids; n3 fatty acid levels were generally higher than those of n6 except in *T. kammalensis* and *T. japonicus*.

C. sinicus and *S. robusta* were the dominant species in the zooplankton. Statistical analyses (one-way ANOVA and Tukey's test) indicated no significant differences between (P>0.05) the fatty acid profiles (Table 2) of copepods and arrow worms. When the shrimp species *A. chinensis* (pelagic), *Metapenaeus joyneri* (benthic), and *S. crassicornis* (benthic) were compared via ANOVA analyses based on the compositions of fatty acids (Table 2), it was found that the fatty acids were similar (F=2.89, P=0.07).

Table 2 Proportions of selected fatty acids (FA) in all samples

	Calanus sinicus	*Sagitta crassa*	*Acetes chinensis*	*Metapenaeus joyneri Miers*	*Solenocera crassiocornis*	*Thrissa kammalensis*	*Trachurus japonicus*	*Harpodon nehereus*	*Pseudosciaena polyactis*	*Trichiurus haumela*
	(*m*=3)	(*m*=2)	(*m*=2)	(*m*=3)	(*m*=3)	(*n*=4)	(*n*=3)	(*n*=6)	(*n*=35)	(*n*=17)
C15:0	0.47±0.13	0.64±0.11	0.68±0.06	0.64±0.02	1.01±0.11	0.60±0.03	0.86±0.09	0.57±0.03	0.65±0.12	0.49±0.15
C16:0	12.66±0.86	17.77±0.19	23.38±1.46	18.96±1.09	13.51±0.83	32.37±1.23	32.18±2.42	23.25±2.47	23.77±1.17	22.01±1.72
C17:0	0.71±0.04	0.75±0.03	1.51±0.25	1.98±0.11	2.56±0.32	1.05±0.02	1.43±0.46	1.19±0.64	1.22±0.28	1.47±0.19
C18:0	3.70±0.89	5.13±0.27	5.25±0.08	8.83±0.46	7.13±0.37	8.31±1.44	15.99±5.37	5.55±1.81	6.23±1.58	7.77±1.21
C16:1n7	6.49±0.37	9.92±0.47	8.07±0.77	6.68±0.33	4.53±0.48	8.48±0.63	8.46±1.56	11.07±4.16	8.36±3.40	3.70±1.62
C18:1n9	5.81±0.11	3.75±0.10	7.66±0.27	10.17±0.78	7.95±0.20	15.50±1.25	7.18±1.65	7.57±1.61	9.36±4.31	14.29±2.99
C18:1n7	nd	0.96±0.04	3.77±0.02	3.97±0.55	5.36±0.32	8.21±1.65	12.89±0.49	5.29±1.33	6.48±0.77	2.78±0.27
C18:2n6	1.88±0.13	2.53±0.20	2.14±0.07	1.78±0.08	0.55±0.06	0.53±0.08	1.30±0.84	2.17±0.76	2.14±0.77	0.77±0.16
C20:1n9	3.04±0.10	3.23±0.08	2.61±0.03	0.65±0.17	0.87±0.02	4.01±0.25	0.88±0.17	1.10±1.12	0.75±0.52	0.84±0.57
C22:1n11	7.75±0.68	4.78±0.11	1.73±0.07	0.86±0.30	nd	5.99±0.87	1.27±0.32	1.89±1.28	1.04±0.44	2.07±0.43
C20:4n6	nd	nd	2.34±0.30	6.94±0.55	8.69±0.31	0.68±0.52	1.34±0.0.49	1.78±0.91	2.44±0.96	2.97±0.92
C20:5n3	10.56±0.15	10.30±0.17	12.44±0.82	12.26±0.63	13.55±0.45	1.56±1.03	1.66±1.47	6.98±1.34	8.04±0.93	5.30±0.44
C22:6n3	20.42±0.07	26.96±1.08	16.69±1.87	14.83±1.38	15.71±1.30	1.48±1.09	3.58±3.66	17.88±5.52	18.29±6.30	25.47±7.57
SFA	25.36±1.71	30.20±0.10	33.04±2.02	32.22±1.66	26.07±1.28	48.71±1.74	57.16±7.32	36.70±2.88	35.66±1.74	35.12±2.40
MUFA	23.76±0.38	25.73±0.68	24.60±0.92	22.33±0.59	19.41±0.67	42.23±4.03	30.97±3.17	28.16±5.49	26.78±8.58	23.49±5.29
PUFA	50.88±1.33	44.07±0.58	42.35±2.94	45.44±2.25	54.52±1.93	9.05±3.34	11.87±6.97	35.14±7.22	37.56±7.98	41.39±7.41
n-3	38.64±0.59	40.60±0.92	36.25±2.59	33.28±1.57	36.25±1.10	5.53±2.98	7.14±5.86	29.69±6.77	30.88±6.28	36.33±6.49
n-6	12.24±0.87	3.47±0.34	6.10±0.35	12.16±0.68	18.27±0.87	3.53±0.43	4.73±1.34	5.40±1.31	6.69±1.86	5.06±1.10

Values are mean±SD. SFA: saturated fatty acids; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; n-3: *n*3 series PUFA; n-6: *n*6 series PUFA. *m*: number of analysis; *n*: number of fish samples.

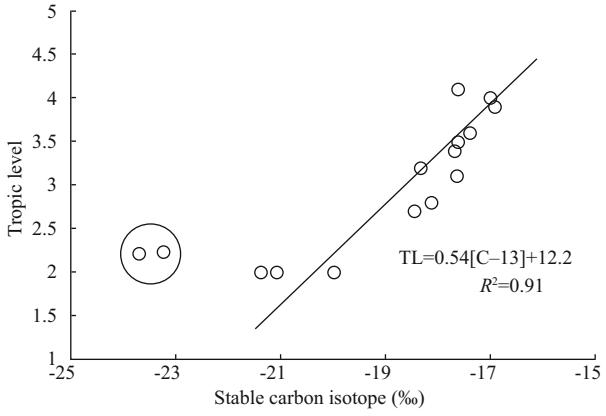

Fig.1 Corrected stable carbon isotope values by a lipid normalizing model versus trophic level

3.2 Compound-specific stable carbon isotopes of fatty acids

Table 3 lists the $\delta^{13}C$ values of compound-specific isotopes of fatty acids from various species. Corrected $\delta^{13}C$ values of total organic carbon ranged from -23.52‰ to -16.92‰, similar to values observed in a previous study (Wan et al., 2010). The relationship of corrected stable carbon isotopes of total organic carbon with trophic level is expected to be linear, except for some benthic species (Fig.1). Carbon isotopes displayed a stepwise enrichment of about 1.7‰–2‰ with each trophic level, in contrast with the generally held view that there is minimal enrichment or fraction of carbon isotopes within food webs (0‰–1‰) (Michener and Schell, 1994; McCutchan et al., 2003).

Compared with stable carbon isotopes of total organic carbon, all fatty acid ^{13}C values were depleted with respect to the total. This finding is consistent with the relative depletion in ^{13}C that occurs during lipid biosynthesis compared with other biochemical pathways (Jim et al., 2003). Figure 2 shows the difference in $\delta^{13}C$ values between total organic carbon and individual fatty acids (C16:0 and C22:6n3). Most pelagic species had a smaller difference between isotopes of total organic carbon and C16:0 than between isotopes of total organic carbon or C22:6n3. The average differences between the various compounds and total organic carbon in these species ranged from 4.1‰ to 8.4‰. The average value for the difference among various compounds was 6.2‰, which is close to the definition value (6‰) for the

Table 3 $\delta^{13}C$ values of fatty acids of various species (mean±SD)

	Calanus sinicus	*Sagitta crassa*	*Acetes chinensis*	*Metapenaeus joyneri Miers*	*Solenocera crassicornis*	*Thrissa kammalensis*	*Trachurus japonicus*	*Harpodon nehereus*	*Pseudosciaena polyactis*			*Trichiurus haumela*			
									7 cm	9 cm	11–13 cm	16.5–17 cm	42 cm♂	58 cm♀	58 cm♂
	(*m*=4)	(*m*=4)	(*m*=3)	(*m*=1)	(*m*=4)	(*n*=4)	(*n*=4)	(*n*=2)	(*n*=2)	(*n*=2)	(*n*=4)	(*n*=4)	(*n*=3)	(*n*=3)	(*n*=3)
C15:0	-26.70 ±0.27	-28.15 ±0.33	-27.04 ±0.26	-24.43	-22.74 ±0.35	-23.50 ±0.10	-24.36 ±0.31	-24.38 ±0.21	-20.24 ±0.00	-20.96 ±0.53	-22.72 ±0.98	-24.84 ±0.38	-26.76 ±0.00		-25.77 ±0.32
C16:0	-27.32 ±0.26	-25.51 ±0.08	-26.99 ±0.21	-24.36	-23.50 ±0.14	-21.81 ±0.40	-22.19 ±0.10	-21.89 ±0.30	-23.19 ±0.00	-22.05 ±0.02	-21.57 ±0.15	-23.60 ±0.05	-24.41 ±0.37	-24.04 ±0.09	-24.84 ±0.11
C17:0	-25.58 ±0.36	-26.31 ±0.61	-23.89 ±0.30	-23.73	-23.00 ±0.38	-24.23 ±0.31	-24.08 ±0.31	-22.76 ±0.08	-21.86 ±0.00	-22.42 ±0.06	-21.87 ±0.76	-24.78 ±0.38	-24.26 ±0.00		-25.42 ±0.74
C18:0	-26.03 ±0.40	-24.80 ±0.32	-25.74 ±0.15	-23.40	-22.83 ±0.34	-21.05 ±0.32	-22.04 ±0.11	-20.26 ±0.00	-20.97 ±0.11	-21.85 ±0.21	-21.24 ±0.48	-23.33 ±0.18	-23.72 ±0.09	-23.19 ±0.61	-24.16 ±0.19
C16:1n7	-26.35 ±0.49	-25.99 ±0.11	-26.73 ±0.79	-24.45	-23.63 ±0.12		-24.04 ±0.29	-25.09 ±0.28	-24.76 ±0.00	-25.03 ±0.05	-24.44 ±0.19	-25.45 ±0.16	-26.08 ±0.35	-26.89 ±0.00	-25.84 ±0.55
C18:1n9	-28.78 ±0.70	-28.08 ±0.33	-29.19 ±0.56	-24.82	-23.93 ±0.26	-20.81 ±0.41	-23.19 ±0.65	-22.27 ±0.48	-23.95 ±0.06	-23.46 ±0.21	-23.35 ±0.53	-25.87 ±0.23	-25.17 ±0.85	-26.88 ±0.13	-27.67 ±0.58
C18:1n7	-29.85 ±0.48	-27.13 ±0.51	-26.61 ±0.40	-24.93	-23.55 ±0.30	-21.50 ±0.41	-21.85 ±1.26	-22.11 ±0.32	-23.98 ±0.02	-23.50 ±0.18	-23.23 ±0.11	-23.82 ±0.28	-24.55 ±2.09	-25.89 ±1.11	-22.17 ±0.42
C18:2n6	-27.16 ±0.50	-26.24 ±0.59	-27.51 ±0.32	-25.88	-23.56 ±0.24	-21.03 ±0.89	-23.54 ±0.43	-21.45 ±0.26			-22.30 ±0.76	-24.96 ±0.23	-25.03 ±0.00	-25.34 ±0.00	-26.32 ±0.54
C20:1n9	-23.19 ±0.88	-25.21 ±0.75	-25.77 ±0.08	-24.18	-25.00 ±0.43	-20.68 ±0.26	-22.11 ±0.58				-21.09 ±0.48	-24.02 ±0.29	-27.52 ±0.00		-24.39 ±0.22
C22:1n11	-24.03 ±0.92	-24.65 ±1.02	-24.27 ±0.81				-19.82 ±1.08					-22.11 ±0.15			-23.04 ±0.63
C20:4n6			-27.02 ±0.00	-26.61	-26.37 ±0.36	-24.13 ±0.13	-26.72 ±0.34	-25.01 ±0.09	-24.14 ±0.09	-25.13 ±0.04	-25.83 ±0.15	-28.75 ±0.33	-26.21 ±0.55		-29.00 ±0.20
C20:5n3	-31.67 ±0.37	-30.18 ±0.22	-25.73 ±0.43	-27.21	-28.16 ±0.31	-23.71 ±0.28	-27.55 ±0.20	-27.94 ±0.12	-26.11 ±0.23	-28.07 ±0.07	-25.63 ±0.45	-29.22 ±0.28	-28.20 ±0.00		-28.47 ±0.24
C22:6n3	-30.13 ±0.47	-30.32 ±0.10	-28.94 ±0.23	-28.60	-27.34 ±0.66	-29.52 ±0.10	-24.86 ±0.47	-23.99 ±0.12	-24.00 ±0.02	-24.28 ±0.31	-25.50 ±0.27	-27.70 ±0.16	-28.96 ±1.63	-29.08 ±0.41	-27.64 ±0.09
$\delta^{13}C$	-21.4	-20	-22.43	-23.27	-23.72	-23.52	-17.71	-17.64	-18.48	-18.14	-17.66	-17.42	-16.92	-17.03	-17.64

Stable carbon isotopes of total organic carbon are estimated by arithmetic correction techniques (Wang et al., 2009); *m*: number of analysis; *n*: number of fish samples.

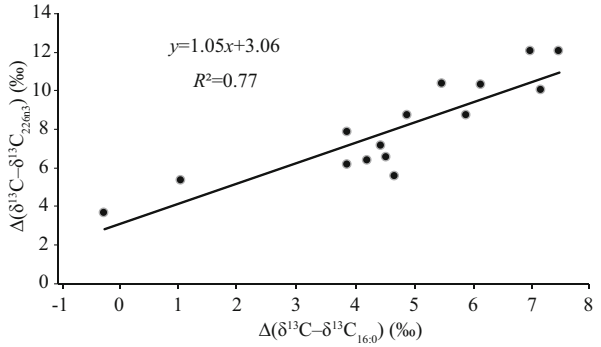

Fig.2 Difference in bulk carbon isotopes with individual compounds (C16:0 and DHA) among various species

depletion of ^{12}C (‰) previously found in lipids relative to proteins during the arithmetic correction for stable isotopes of total organic carbon in food-web studies using a lipid-normalizing model (Wang et al., 2009). The $\delta^{13}C$ values of 15:0 and 17:0 were relatively close in fish samples. In most cases, EFAs had more negative $\delta^{13}C$ values than other fatty acids in various species. The $\delta^{13}C$ values of EPA were heavier than DHA in fish samples.

Unlike fatty acid composition, ANOVA analysis of $\delta^{13}C$ values for compound-specific isotopes of fatty acids revealed significant differences among species (F=6.35, P=0.004). Table 3 compares $\delta^{13}C$ values of all fatty acids within animals of the same species (*P. polyactis*) but of different lengths, and found a remarkable difference between individual of >13 cm in length and those of <13 cm in length (F=2.29, P=0.033).

Compound-specific stable carbon isotopes have

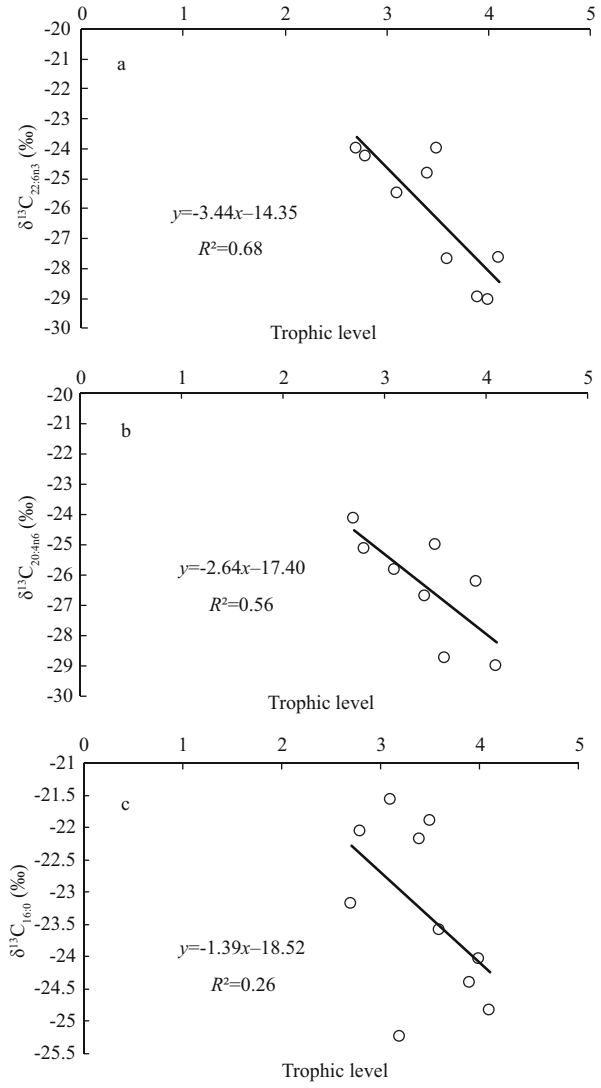

Fig.3 Compound-specific isotopes versus trophic level

a. C22:6n3; b. C20:4n6; c. C16:0.

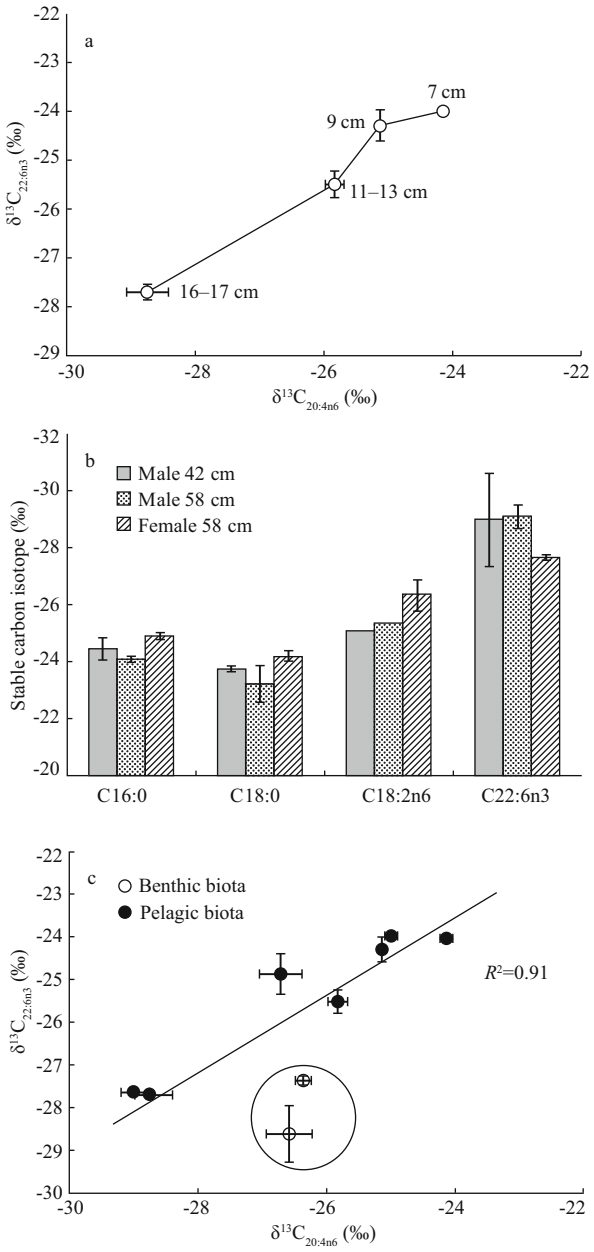

Fig.4 Variation in compound-specific isotopes with body length (upper/lower limits), gender and lifestyle habit

a. distribution of compound-specific isotopes of C22:6n3 and C20:4n6 in *P. polyactis* of different body lengths; b. variation in the compound-specific isotopes of selected fatty acids in *Trichiurus haumela*; c. distribution of compound-specific isotopes of C22:6n3 and C20:4n6 among pelagic and benthic species.

been used previously in food-web studies (Budge et al., 2008). Figure 3 shows the relationships of individual isotopes of fatty acids among higher trophic level species. PUFAs (such as 20:4n6 and DHA) are correlated with trophic level, while isotopes of 16:0 have no significant relationship with trophic level. Figure 4a shows the variability for individual fatty acid isotopes of *P. polyactis* of various lengths. There was a large difference between the isotopes of C20:4n6 and DHA within the <13 and 16–17-cm length groups. However, in *T. haumela*, the differences between fish of different lengths were not significant; however, compositions were slightly different between the sexes (Fig.4b). Figure 4c illustrates the differences in C20:4n6 and DHA isotopes among benthic species (*M. joyneri* and *S. crassiocornis*) and pelagic species (such as *T. kammalensis*). There was a good correlation for C20:4n6 and DHA among pelagic species from various trophic levels. Higher trophic level species had more negative isotope compositions. The compositions of C20:4n6 and DHA in benthic species were more negative than those of pelagic species at the same trophic level.

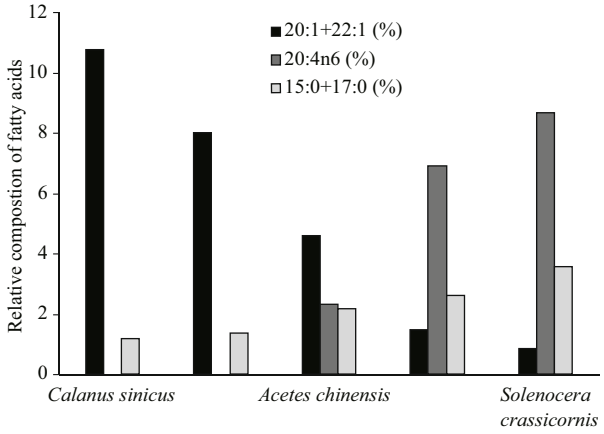

Fig.5 Relative composition of fatty acids from selected species in lower trophic levels from zooplankton to *Acetes chinensis*, *Metapenaeus joyneri*, *Solenocera crassicornis*

20:1+22:1: the sum of C20:1+C22:1; 15:0+17:0: the sum of C15:0+C17.

4 DISCUSSION

4.1 Fatty acid biomarkers and stable isotopes in the food web

In an attempt to understand trophic relationships, it is increasingly common for researchers to use fatty-acid composition to trace the transfer of organic matter through aquatic food webs (Graeve et al., 2002; Iverson et al., 2002). A number of specific fatty acids or ratios are only found in certain taxa, meaning that they can be used to trace specific organisms (Alfaro et al., 2006). Figure 5 shows how relative amounts of some fatty acids can be used to identify various food sources, e.g., diatoms (20:5n3, 16:1/16:0), dinoflagellates (22:6n3) or copepods (C22:1+C20:1) (Alfaro, 2008). Fatty acid profiles of food sources (i.e., copepods and arrow worms) and low trophic level species are shown in Fig.5, and one-way ANOVA results for fatty acid biomarkers indicate that there were no significant differences among those species (P>0.05). A higher calanoid copepod contribution (the sum of C22:1+C20:1) was indicated for *A. chinensis*.

C20:4n6 is considered to be a marker of macroalgae (primary producers in freshwater and terrestrial ecosystems) (Iverson, 2009), which can be transferred via a detritus-based food chain (Fukuda and Naganuma, 2001). Higher C20:4n6 levels in benthic species suggested the importance of a detritus-based food source to those species. A small bacterial source of fatty acids (C15+C17) was detected at ~1%–2% in most species but at >2% in benthic species, which may be derived from dietary sources and/or the metabolism of commensal bacteria in the intestine (Arts and Wainman, 1999). Seasonal changes in the major fatty acids and lipid ratios have been observed in deep-sea crustaceans in the Mediterranean, which suggests greater dietary diversity with temporal variation (Cartes, 2011). In this study, all biota were sampled in one season, but care is still required when those parameters are applied for direct comparison.

Tracing food source is more complex in fish at higher trophic levels because of limited fatty acids: the same fatty-acid markers within various organisms are difficult to identify as material from different producers through food webs (Dalsgaard et al., 2003). Statistical analyses of the entire fatty acid profile may resolve this problem (Jim et al., 2003). Zooplanktivores (such as *Acetes chinensis* and *T. kammalensis*) and benthivores can be separated from piscivores (e.g., *T. haumela*).

In general, animals could synthesize fewer and simpler fatty acids (such as 14:0, 16:0 and 18:0 and their monounsaturated isomers 14:1n5, 16:1n7 and 18:1n9) by inserting a double bond at the ninth carbon from the carboxyl end. Calanoid copepods are believed to have an unusual ability to produce large amounts of long-chain monounsaturated fatty alcohols (e.g., 20:1n9, 22:1n11) as part of their primary storage of fats which may be traceable in higher trophic level organisms (Sargent and Henderson, 1986). However, polyunsaturated fatty acids, especially EFAs, are believed to be supplied to animals in their diet, although some animals can synthesize them when sufficient quantities of 18:2n6 and 18:3n3 precursors are available (Sprecher, 2000).

It is noticeable that higher trophic fractionation (1.7‰–2‰) was observed in the present study than in other studies. Stable carbon isotopes corrected by lipid normalization were employed (Wang et al., 2009), which will modify the original values, making them a little heavier. Because the C/N ratio is also applied during the correction, the deviations differed for various species, making the fraction a bit larger than expected. If trophic fractionation of stable carbon isotopes was calculated for fish samples alone, the value would be about 1‰, which is close to traditional observations (Van der Zander et al., 2001; Post, 2002). There may be several explanations for these observations. First, trophic levels of the marine food web in China lack systematic long-term studies with, for example, different definitions of trophic levels being used (Deng et al., 1986; Wei and Jiang, 1992;

Zhang and Tang, 2004). Thus, these differences can cause some confusion in studies of trophic levels. Second, estimations of trophic level are based on gut content analysis, which assumes that all gut contents are identifiable and exactly quantifiable, and are of known trophic levels. The necessary conditions are not easy to satisfy during sample analysis, causing errors in the estimation of the trophic level (Dou, 1996). Third, the results of a recent study were employed, but the estimation of trophic level was based on a 2000–2001 survey. In addition, it has been reported that trophic levels display annual fluctuations and shifts that could not be avoided in the East China Sea food-web study (Zhang and Tang, 2004). As a result of the depleted values for organic detritus in the sediments, the isotopic composition of two benthic species was more depleted than that of pelagic species as illustrated in Fig.1. In conclusion, stable carbon isotopes were found to be correlated with trophic level in the present study but were not efficient to elucidate the interactions within the food web.

4.2 Compound-specific stable carbon isotopes and trophodynamics

The composition of compound-specific stable isotopes can demonstrate the relative importance of routing and de novo synthesis for each fatty acid. The 16:0, 18:0, 18:1n9, 20:1n7 and 22:1n9 fatty acids are non-essential fatty acids (non-EFAs) that can be synthesized de novo by the Δ9 desaturase enzyme, which inserts a double bond at the ninth carbon from the carboxyl end (Jim et al., 2003). The variation in non-EFA carbon isotopes among different species has a smaller range, which may be due to their utilization of similar organic materials and the same biosynthetic process. It is noted that the $\delta^{13}C$ values of bacteria-specific fatty acids (*iso*-C15:0 and *iso*-C17:0) were similar for most species, with average values of approximately -24‰. It has been reported that the isotopic fractionation between the substrate and bacteria-specific fatty acid is relatively constant (-4‰ to -6‰) (Teece et al., 1999). Thus, it can be speculated from the results of the present study that bacteria mainly utilize marine organic matter (F07E-18‰).

EFAs have been identified as key nutrients that cannot be synthesized by organisms at rates sufficient to meet their basic biochemical requirements and, thus, must be obtained largely through the diet (Arts and Wainman, 1999; Kainz et al., 2004). The PUFAs 20:4n6, 20:5n3 and 22:6n3 were considered to be EFAs in the present study. PUFAs are produced by a number of different classes of phytoplankton, but there is some evidence that they also can be produced by chain elongation and desaturation of precursors, such as 18:3n3, in higher vertebrates (Cook, 1996). For example, 22:6n3 could have a longer pathway of synthesis via enzyme processes and its carbon isotope composition might be more negative than that of 20:5n3.

It is important to bear in mind that, in nature, fatty acids rarely exist in a free form; in both primary producers and consumers, both EFAs and non-EFAs are generally incorporated as part of a compound. The majority of these are the main constituents of triacylglycerols (TAGs), wax esters (WEs) and phospholipids. It has been reported that ingested TAGs are hydrolyzed (ester bonds broken) in the gut by lipases and esterases to their component fatty acids (chain lengths and double bond positions are generally conserved), monoacylglycerol and glycerol (Budge et al., 2006). After digestion, certain fatty acids (FAs) are reduced to their corresponding fatty alcohols and then incorporated along with other ingested FA into WEs for storage (Sargent and Henderson, 1986). The fraction of carbon isotopes of individual compounds is shown in Fig.3. In contrast to the enrichment of carbon isotopes of bulk organic matter in fish samples, negative “bioaccumulation” of stable carbon isotopes of EFAs was observed in higher trophic level samples. Higher-level organisms have more negative carbon isotope values, which may reflect both the fraction and the variable diet available to them (Ruess et al., 2005). Similar results have been reported from an Arctic marine food-web study (Budge et al., 2008). As a result of the various effects of biochemical processes on carbon isotope discrimination, in the present study, a general pattern could not be assigned to trophic transfer from the bottom to higher levels of the food web.

The isotopic composition in organisms is correlated with diet and body condition. As shown in Fig.4, the isotopic composition of 22:6n3 and 20:4n6 in little yellow croakers (*P. polyactis*) of a larger body size has shifted to more negative values compared with smaller sized individuals. The shift in diet from a shrimp+fish mixture to fish alone is the main reason for this type of distribution (Lin, 2007). Although the fatty acid composition was found to be consistent in samples of large-head hairtails (*T. haumela*) with respect to sex and length, the isotopic composition, especially of EFAs, was distinctly different between the two sexes, which may be related to their physiology.

As shown by the results of stable carbon isotope and fatty acid composition analyses, there were some clear differences between benthic and pelagic species (Fig.2 and Table 2). Analysis of compound-specific isotopes was warranted because the isotope data could provide more definitive information, especially for PUFAs (C20:4n6 and C22:6n3) (Fig.4c). In this study, the benthic species were distributed mainly at the sediment-water interface between 0 and 1 m above the bottom where they exploit fresh food, deposited/resuspended particulate organic matter, small invertebrates and other crustaceans (Cartes et al., 2008). A comparative study of zooplankton and suprabenthos in the Algerian Basin revealed a different source of carbon based on stable carbon isotopes and a more complex food web in the near-bottom water column (Fanelli et al., 2009). The diet of benthic species contains more degraded items whose carbon isotopes are derived from a large biochemical fraction (Pearson et al., 2003). The potential contribution from terrestrial organic matter to the diet of benthic species could also result in negative values. Pelagic species have a more stable relationship between isotopes of C20:4n6 and DHA than benthic species, whose diets may be more complex (Fig.4c).

Because material and energetic relationships within marine ecosystems can have fundamental implications for fisheries resources, especially in periods of global climate change, knowledge of feeding history is important in evaluating ecosystem performance. This study highlights the importance of using a combination of complementary tools to identify feeding variations. Gut contents analysis is still an excellent method of quantifying ingested food (Alfaro, 2008). However, fatty acids/compound-specific isotopes of fatty acids are especially useful for detritivores and smaller organisms that can be analyzed whole. This can partly resolve the problem of potential variations in fatty acids in different tissues but also indicates the necessity for fatty acids profile analysis, with increased replicates as in gut content analysis.

5 CONCLUSION

This study of compound-specific isotopes of fatty acids in the East China Sea ecosystem is a promising start in elucidating the interactions among various trophic levels. Analysis of isotopes of PUFAs may be a valuable tool in identifying changes that more established methods (such as isotopes of total organic carbon or composition of fatty acids) might not detect, such as dietary shifts or differences between the sexes. Various dietary structures could be traced from molecular isotopes of selected fatty acids in the Shiba shrimp (*Matapenaeus joyneri*), the coastal mud shrimp (*Solenocera crassicornis*) and the northern Maoxia shrimp (*Acetes chinensis*). The first two shrimp species are mainly benthos feeders, while *A. chinensis* is a pelagic species, although they have similar fatty acid compositions. A good correlation was found for the isotopes of arachidonic acid (C20:4n6, ARA) and docosahexaenoic acid (C22:6n3, DHA) among pelagic species from higher trophic levels. The isotopic compositions of DHA in benthic species were more negative than those of pelagic species at the same trophic level. However, these tools must be used with caution because isotopic fractionation may occur and could be affected by factors such as dietary composition and trophic level. Variations among samples (sex, body size, physiological status and other factors) may also exert an impact on isotopic values. Increasing the number of samples analyzed will reduce potential bias, while systematic studies, in combination with statistical analysis, could help reduce bias and errors in data interpretation. The quantitative estimation of the diet of predatory species is perhaps of greatest interest in current studies using fatty acids to elucidate food-web associations (Iverson, 2009). Analysis of compound-specific isotopes of fatty acids is a promising tool for investigating the contribution of various foods/diets to higher trophic level organisms and for tracking carbon flow through marine food webs.

6 ACKNOWLEDGEMENT

The authors thank the Marine Biogeochemistry Group for help and support during field and laboratory analyses. The authors acknowledge the helpful comments and suggestions of the anonymous reviewers.

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