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Potential dietary influence on the stable isotopes and fatty acid composition of migratory anchovy (*Coilia mystus*) around the Changjiang Estuary

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*The stable carbon and nitrogen isotopes and fatty acid composition of tapertail anchovy (*Coilia mystus*) at four migration stages collected around the Changjiang Estuary were analysed to investigate the variations in the trophic biomarkers during the fish migration. δ^{13} C and δ^{15}N values of* C. mystus *ranged from −21.5 to −15.4‰ and from 6.9–15.8‰, respectively. Both δ^{13}C and δ^{15}N were enriched during migration. Polyunsaturated fatty acids were the dominant fatty acids and the major fatty acids found in* C. mystus *were C20:5*n*-3, C22:6*n*-3, C20:4*n*-6, C16:0, C18:0, C16:1*n*-7, C18:1(*n*-9,* n*-7) and C20:1 + C22:1. Significant changes among* C. mystus *at different migration stages were found both in the fatty acid composition and specific fatty acid concentration. Though the enrichment of stable isotopes may due to multiple factors (e.g. diet shift, environment and ontogeny), the dietary influence can be determined by the variation in fatty acid composition. Changes in the concentrations of benthic markers (C18:1*n*-7 and C20:4*n*-6) and pelagic markers (C18:1*n*-9 and C20:1 + C22:1) in* C. mystus *during the migration may suggest that benthic and pelagic food sources alternately dominated the anchovies' diet during different migration stages. It seems that application of multiple biomarkers in the trophic study of migratory fish will elevate the reliability of the analysis.*

Keywords: migratory fish, diet source, estuary, multiple biomarkers

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INTRODUCTION

Stable carbon and nitrogen isotope ratios as trophic biomarkers have great advantages over traditional gut content analysis for both freshwater and marine fish trophic ecology studies by providing information on the long-term assimilation items (Sugisaki & Tsuda, 1995; Gu *et al.*, 1996; Vander Zanden & Vadeboncoeur, 2002; Bardonnet & Riera, 2005; Harrod *et al.*, 2005). The contributions of prey to a predator's diet can be estimated using a model if all potential prey are collected and significant differences exist among prey types in their stable isotope patterns (Post, 2002; Pitt *et al.*, 2009). However, in most cases, it is difficult to collect all potential diet items, and there are also cases where the stable isotope patterns of different diet items overlap. Therefore, fatty acid composition has been used, together with stable isotopes, to trace the transportation of organic materials through the food web (Sugisaki & Tsuda, 1995) and to identify trophic interactions within the food web (Budge *et al.*, 2001; Alfaro *et al.*, 2006; Hessen & Leu, 2006; Maazouzi *et al.*, 2007; Alfaro, 2008; Stowasser *et al.*, 2009a, b; Wan *et al.*, 2010).

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Anadromous and catadromous fish are characterized by their extensive migrations between the open sea and fresh water (Nybakken, 1997). According to previous studies on wild individuals and feeding experiment in the laboratory, the biomarkers of migratory fish were plastic, and changes in the habitat or food were both followed by variation in the patterns of both stable isotopes and fatty acid composition (Bardonnet & Riera, 2005; Ciancio *et al.*, 2008; Prigge *et al.*, 2012). Generally, oceanic organic matter is characterized by enriched δ^{13}C and terrigenous organic matter is comparatively depleted in δ^{13}C (Bardonnet & Riera, 2005). The difference in the δ^{13}C patterns of the habitats may make it possible to discriminate the diet source when they shift between the two kinds of habitats. It has been observed that the changes in δ^{13}C of the migratory fish were in accord with the δ^{13}C pattern of the habitat (Bardonnet & Riera, 2005; Ciancio *et al.*, 2008). For instance, increases were found in δ^{13}C values of anadromous rainbow trout (*Oncorhynchus mykiss*) after migration (Ciancio *et al.*, 2008). The δ^{13}C values of European eels (*Anguilla anguilla*) decreased during their migration to a river (Bardonnet & Riera, 2005). However, the δ^{15}N values of both species increased during their respective migrations (Bardonnet & Riera, 2005; Ciancio *et al.*, 2008). The observed increase in the polyunsaturated fatty acids concentrations of salmon after their migration to the ocean could be due to their dietary changes or to physiological adaptability, because when fish

migrate from fresh water to salt water, they will adjust their fatty acid composition as a physiological adaptation (Saddler *et al.*, 1972). It seems that both stable isotope profiles and fatty acid composition of migratory fish are under the influences of multiple factors including diet composition, location and ontogeny of individuals (Saddler *et al.*, 1972; Bardonnet & Riera, 2005; Harrod *et al.*, 2005; Ciancio *et al.*, 2008), which will bring challenges in the applicability of the biomarkers to the trophic study of migration species. There are several studies that reported the variation in biomarkers of two well-known migratory fish, rainbow trout and European eels (Bardonnet & Riera, 2005; Harrod *et al.*, 2005; Ciancio *et al.*, 2008), but in view of the combined influences from multiple factors, shift in the diet sources and trophic levels during their migration period are still not well identified.

Tapertail anchovy (*Coilia mystus*) is a well-known estuarine migratory species in China and widespread in Chinese coastal waters (He *et al.*, 2008). In the Changjiang Estuary fishery in China, it provides approximately 48.6% of the total fish and shrimp catch and became one of the most important commercial species in this area when the total fishery yield decreased in recent years (Liu *et al.*, 2004; He *et al.*, 2008). Besides the commercial value in the coastal region, *C. mystus* also occupies a crucial ecological position. It preys predominantly on zooplankton, and is preyed upon by predators such as hairtail (*Trichiurus lepturus*), little yellow croaker (*Pseudosciaena polyactis*) and white spotted conger (*Conger myriaster*) (Zhou *et al.*, 2004). The Changjiang Estuary and Hangzhou Bay are two important spawning sites for *C. mystus* inhabiting around the Changjiang Estuary, and the Zhoushan Islands offshore are its primary overwintering site (Zhou *et al.*, 2004), as shown in Figure 1. In spring, adult fish gather and migrate to estuarine brackish water to spawn (Yuan & Qin, 1984; Zeng & Dong, 1993; He *et al.*, 2008). Fish larvae graze and grow in the Changjiang Estuary and north Hangzhou Bay (Ni, 1999). In October and November, when the water becomes cold, the adult population and the juvenile fish start to move offshore and pass the winter in deep waters from December to April of the next year (He *et al.*, 2008). In May, offshore overwintering *C. mystus* gather and migrate back into the estuary area to spawn (Ni, 1999).

Coilia mystus is different from other anadromous migratory species (e.g. salmon) in the migration distance and the habitat. The migration distance of *C. mystus* around the estuary is approximately 200 km, maybe ten times shorter than that of salmon. Further, unlike salmon, *C. mystus* never goes upstream to the freshwater, only spawns in the estuary, so the differences between the biomarker patterns of each habitat may not be as distinct as those of salmon. On the other hand, the estuarine area is characterized by complex carbon sources, making the application of biomarkers in this area very challenging and interesting. Additionally, study on the biomarkers of the short distance migratory fish is very limited. Information about the diet composition of *C. mystus* in different migration stages was not well documented, but it is important for understanding the population dynamics of *C. mystus*. It is unknown how the stable isotopes and fatty acid composition of the fish change during migration and whether dietary influence can be reflected on the biomarker variation. To answer these questions, *C. mystus* individuals were collected at different migration stages and their stable carbon and nitrogen isotope patterns and fatty acid composition were analysed. Then the diet sources among different migration stages of *C. mystus* around the Changjiang Estuary were inferred from variation in the two biomarkers.

MATERIALS AND METHODS

Sample collection

Coilia mystus in various migration stages were captured from three habitats (i.e. the Changjiang Estuary, Hangzhou Bay and the offshore Zhoushan Islands) in 2008 and 2009, by the fixed net (25 m width, 5 m height, mesh size 2.5–12 cm) and bottom trawl (7.5 m width, 3.5 m height, mesh size 2.5–12 cm), whose mesh size gradually narrowed from the opening to the end. Samples in four different migration stages, which were labelled M1 to M4, denoting growth stage, overwintering stage, pre-spawning stage and spawning stage, were collected from five stations (HZB1, DS, JT, HZB2, CXI), as shown in Figure 1. The samples were identified by their migration stage and the station where they were collected.

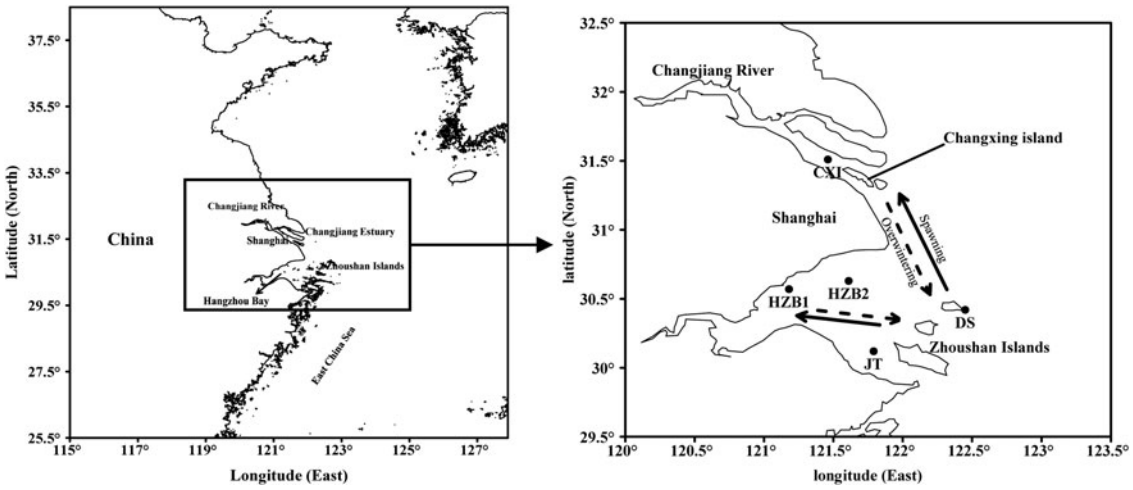

Fig. 1. Sampling locations for *Coilia mystus* around the Changjiang Estuary in China. Migration routes are labelled with an arrowed line. Solid and dotted arrowed lines imply spawning and overwintering migration routes, respectively.

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The sampling station, date, and migration stages of the collected *C. mystus* are shown in Table 1. Juvenile samples in growth stage (M1-HZB1) of *C. mystus* were collected from nearshore waters of the Hangzhou Bay in September 2008. Overwintering stage samples (M2-DSa) were collected north-east offshore from the Zhoushan Islands in March 2009. Pre-spawning stage *C. mystus* were collected from the waters offshore north-east (M3-DSb) and the south-west (M3-JT) of the Zhoushan Islands in May 2009. *Coilia mystus* in the spawning stage were sampled near the Changxing Islands in the Changjiang Estuary (M4-CXI) and the north of Hangzhou Bay (M4-HZB2) in June 2009. Standard length and body weight were measured following collection (Table 1). Pre-spawning stage *C. mystus* collected from DS station (M3-DSb) were separated into four groups according to their standard length, and numbered from I to IV, to investigate whether individuals of different body lengths within one migration stage exhibited stable isotope and fatty acid composition variation.

The diet composition of *C. mystus* has previously been investigated (Yuan & Qin, 1984; Ni *et al.*, 1999; Zhou *et al.*, 2004). Copepods, euphausiaceans, *Acetes chinensis*, decapods (e.g. *Leptochela gracilis* and *Squilla* juveniles), and some small juvenile fish (e.g. *Pseudosciaena polyactis* and *Harpadon nehereus*) have been identified in the gut of *C. mystus* collected offshore from the Zhoushan Islands; euphausiaceans, copepods and *A. chinensis* were the predominant species (Zhou *et al.*, 2004). Accordingly, seston, copepod, and fish larvae samples were collected at the CXI station, and *A. chinensis* was collected at the HZB1 station in the present study. Approximately 1–2 l of water was filtered through a 200 μm net and then filtered on a pre-combusted GFF 47 mm filter (0.7 μm, Whatman, 500°C, 5 h) to obtain the seston sample dominated by the phytoplankton (Pond *et al.*, 1998). Zooplankton were sampled by plankton trawl (diameter 50 cm, mesh size 505 μm), washed with filtered seawater and filtered on a pre-combusted GFF 47 mm filter (0.7 μm, Whatman, 500°C, 5 h). Fish larvae and *A. chinensis* were then removed manually and placed in plastic bags. All samples were frozen immediately and stored at −20°C. In the laboratory, the white dorsal muscle of the fish was cut from the body. White dorsal muscle was used to study the fish's feeding ecology because it was found to be less variable in $\delta^{13}C$ and $\delta^{15}N$ than red muscle and other organs such as the liver and heart (Pinnegar & Polunin, 1999). All samples were lyophilized in a freeze-dryer (LOC-1, Christ, Germany) and stored at −40°C until further analysis. The dorsal muscle was ground into powder before analysis.

Stable carbon and nitrogen isotope analysis

Seston and zooplankton samples were digested with acid (1 M HCl) to remove carbonates and dried at 50°C for 12 h. *Coilia mystus* dorsal muscle was analysed without acidification. Organic carbon and total nitrogen contents were analysed using an elemental analyser (Vario EL III, Elementar, Germany). Stable carbon and nitrogen isotopes were measured with an isotope-ratio mass spectrometer (Finnegan Delt plus XP, Thermo, Germany). Lipids were not removed prior to measurements. The results were normalized to Vienna Pee Dee Belemnite standard (PDB) for $\delta^{13}C$ and the atmospheric N_2 standard (AIR) for $\delta^{15}N$ (Overman & Parrish, 2001); results were expressed in δ notation as δX (‰) = $((R_{sample}/R_{standard}) - 1) \times 1000$, where $X = {}^{13}C$ or ^{15}N and $R = {}^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$. The precision of $\delta^{13}C$ and $\delta^{15}N$ analyses was ±0.1‰.

Coilia mystus trophic levels were calculated according to the method described in Vander Zanden & Fetzer (2007), and 3.4‰ was used as the $\delta^{15}N$ accumulation coefficient of the marine food web across trophic levels. *Acetes chinensis*, a primary consumer collected from HZB1, was set as the baseline because primary consumers tended to have more steady isotopic ratios than primary producers (Vander Zanden & Fetzer, 2007). The trophic level of primary consumer was set at 2.

Lipid extraction and fatty acid composition analysis

Methods of lipid extraction and fatty acid composition analysis were referred to Cui *et al.* (2012). For dorsal muscle samples, approximately 5 ml of a chloroform and methanol (2:1) solvent was added to each 100 mg sample. For the seston and zooplankton samples, a similar procedure was used except that the weight of the samples used for extraction was adjusted according to their total lipid concentration. Fatty acid data are expressed as a mass percentage of the total fatty acids. The recovery rate of the whole analysis procedure for fatty acids was in the range of 83.3–98.7%. The standard deviations (SDs) of individual fatty acid proportions in replicate analyses were in the range of 0.0–1.5%. Fatty acids were named using a shorthand notation of CA:B *n*-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). If not specifically stated, concentrations of C20:1 and C22:1 are the sum of their *n*-11 and *n*-9 isomers.

Table 1. Sample name, date, station, migration stage, number of fish (N), standard length and weight of *Coilia mystus* collected in 2008 and 2009. SD, standard deviation

Sample name	Date	Station	Migration stage	N	Standard length (mm)		Body weight (g)	
					Range	Average ± SD	Range	Average ± SD
M1-HZB1	16 September 2008	HZB1	M1	50	51–116	66 ± 12	0.56–5.63	1.15 ± 0.85
M2-DSa	5 March 2009	DS	M2	20	64–165	97 ± 33	0.79–19.18	4.16 ± 5.08
M3-DSb	25 May 2009	DS	M3	20	73–167	103 ± 27	1.47–16.38	5.06 ± 4.59
M3-JT	28 May 2009	JT	M3	10	85–128	114 ± 15	3.86–10.04	7.21 ± 2.72
M4-HZB2	5 June 2009	HZB2	M4	10	153–172	164 ± 8	18.38–25.12	20.38 ± 3.18
M4-CXI	11 June 2009	CXI	M4	10	173–188	182 ± 6	20.56–27.48	24.05 ± 2.76

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Statistical analysis

PRIMER 5.0 (Primer-E) and SPSS17.0 (SPSS Inc.) were used to perform the data analysis (Budge *et al.*, 2008; Wan *et al.*, 2010; Prigge *et al.*, 2012). Values of fatty acids reported in the form of mass % of total fatty acids were root-square transformed to achieve normalization in multivariate analysis (Iverson, 2008). Bray–Curtis similarity matrices were calculated and principal component analysis (PCA) was performed to investigate the difference in fatty acid compositions of *C. mystus* among stations and determine which fatty acids accounted for this difference (Loseto *et al.*, 2009). A cluster analysis was performed based on the PCA scores of samples from various stations to separate the samples into several groups. Then, similarity analysis (one-way ANOSIM) was performed to determine whether the difference in stable isotopes and fatty acid composition among the sample groups was significant (Clarke, 1993, Budge *et al.*, 2008). Similarity of percentages (SIMPER) was performed to identify the fatty acids responsible for the difference among sample groups. Relationships between the biomarkers and the body lengths of *C. mystus* were analysed with correlation analysis. One-way ANOVA was performed to determine whether the specific fatty acids of individuals in different stations were different significantly.

RESULTS

Stable isotope signatures of *C. mystus* in various migration stages

The carbon and nitrogen contents of dorsal muscles of *C. mystus* were 44.2 ± 1.7% and 13.7 ± 0.7%, respectively. The C/N ratio ranged from 3.6 to 4.2 (average: 3.8 ± 0.2). The $\delta^{13}C$ values of *C. mystus* were in the range of −21.5 to −15.4‰ (average: −17.5 ± 1.6‰). Values of $\delta^{15}N$ were in the range of 6.9–15.8‰ (average: 9.5 ± 2.2‰). ANOSIM was performed based on the $\delta^{13}C$ and $\delta^{15}N$ values of *C. mystus* samples using PRIMER 5.0. The results (ANOSIM, $R = 0.605$, $P = 0.001$) suggested a significant difference in the stable isotope signatures among stations. Increases were found in both the $\delta^{13}C$ and $\delta^{15}N$ values across the migration stages (Figure 2). The average $\delta^{13}C$ value of *A. chinensis* was similar with that of *C. mystus* at M1-HZB1. *Coilia mystus* at M1-HZB1 were significantly different from those of *C. mystus* at M2-DSa (ANOSIM, $R = 0.809$, $P = 0.003$) and other stations (M3-DSb, M3-JT and M4-HZB2, M4-CXI) (ANOSIM, $R = 0.995$, $P = 0.01$) in $\delta^{13}C$ values. *Coilia mystus* can be separated into three groups according to the $\delta^{15}N$ values. The values of individuals at M1-HZB1, M2-DSa, and M3-JT were the lowest, values for M3-DSb, M4-HZB2 were intermediate, and values of M4-CXI were the highest. Significant differences existed among the three groups (ANOSIM, $R = 0.671$, $P = 0.001$). The average trophic level of *C. mystus* at M1-HZB1 was 3 (Figure 2).

Fatty acid compositions of organisms around the Changjiang Estuary

A large difference existed in the total fatty acid content (TFA) of organisms in the region (Table 2). The seston samples contained the lowest TFA, only 0.2 ± 0.1 mg g^{-1}. The highest

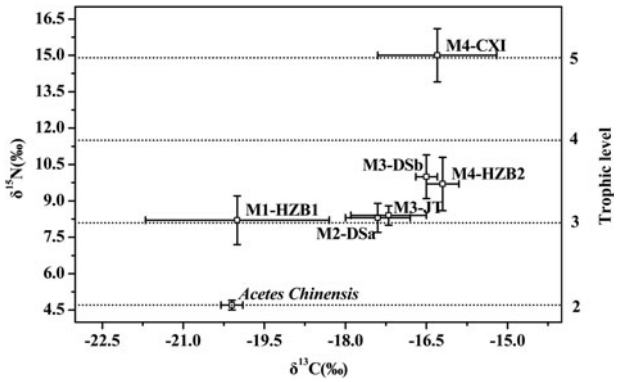

Fig. 2. Dual isotope plot of *Coilia mystus* and zooplankton (*Acetes chinensis*) with trophic levels. Data are presented as the average ± standard deviation.

TFA value was found in fish larvae (46.1 mg g^{-1}). Of all *C. mystus*, those collected at M1-HZB1 contained the lowest TFA, and those from M2-DSa contained the highest TFA. There was no significant difference in the TFA contents of *C. mystus* among different stations except for those at M1-HZB1. The lipid content (mg g^{-1} of dry weight) of *C. mystus* was significantly positively correlated to the TFA ($r = 0.516$, $P = 0.006$).

For seston samples, saturated fatty acids (SFA) were the dominant fatty acids, and the concentration of polyunsaturated fatty acids (PUFA) was extremely low (Table 2). For copepods, fish larvae, *A. chinensis* and *C. mystus*, PUFA was the dominant fatty acid. The seston samples were characterized by higher C16:1*n*-7, C18:1*n*-7 and C18:2*n*-6 + C18:3*n*-3 concentrations compared with zooplankton. In the zooplankton samples, copepods contained the highest C20:1 + C22:1 (>5%) and C22:6*n*-3 concentrations; fish larvae and *A. chinensis* were characterised by high C20:4*n*-6 and C18:1*n*-9 concentrations. For *C. mystus* in all stations, C20:4*n*-6 (Arachidonic, ARA), C20:5*n*-3 (eicosapentaenoic, EPA) and C22:6*n*-3 (docosahexaenoic, DHA) were the dominant essential fatty acids, and they accounted for almost 50% of the total fatty acids. DHA was especially high and accounted for over 30% of the total fatty acids. C16:0 and C18:0 were the dominant SFA, and C16:0 accounted for over 20% of the TFA. The predominant MUFA were C16:1*n*-7 and C18:1 (*n*-9, *n*-7). For *C. mystus* at M3-DSb and M3-JT, the C20:1 + C22:1 concentration was greater than 2%. Regarding the C22:6*n*-3/C20:5*n*-3 (DHA/EPA) ratio, those of copepods, fish larvae and *C. mystus* were higher than 1, but those of seston and the *A. chinensis* samples were lower than 1.

Fatty acid composition variation of *C. mystus* in different migration stages

A PCA was performed based on the fatty acid compositions of *C. mystus* using PRIMER 5.0. The first two PCAs accounted for 63.0% of the variance (Figure 3). The first PC axis on the score plot separated the fishes by placing *C. mystus* from HZB1 on the negative side of PC1 and the samples from other stations on the positive side (Figure 3A, PC1 48.9% of variance explained). The second PC separated DSa from the other stations and accounted for 14.1% of the variance (Figure 3A). According to the results of the cluster analysis

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Table 2. Fatty acid composition of *Coilia mystus* and potential prey items (mass % total fatty acids). Values are shown in average ± standard deviation if more than one sample was analysed (n.d., not detected). N, number of samples used in the analysis; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; *n*-3, sum on unsaturated fatty acids with the first double bond starting after the third carbon atom relative to the terminal methyl group; *n*-6, sum of unsaturated fatty acids with the first double bond starting after the sixth carbon atom relative to the terminal methyl group; TFA, total fatty acids (unit: mg g^{-1}). Copepods, fish larvae and Acetes chinensis are pooled samples with more than 10 individuals, respectively, and are labelled with p.s. in the table.

Fatty acids	Potential prey				*Coilia mystus*					
	Seston N = 4	Copepods p.s.	Fish larvae p.s.	*Acetes chinensis* p.s.	M1-HZB1 N = 50	M2-DSa N = 20	M3-DSb N = 20	M3-JT N = 10	M4-HZB2 N = 10	M4-CXI N = 10
SFA										
C14:0	3.7 ± 0.1	1.9	1.0	1.5	0.7 ± 0.1	0.6 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	0.9 ± 0.2
C15:0	1.0 ± 0.1	0.4	0.5	0.5	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
C16:0	26.3 ± 0.5	14.2	25.7	20.8	26.6 ± 0.4	22.8 ± 1.3	22.6 ± 0.4	22.5 ± 1.2	24.9 ± 0.6	24.1 ± 0.1
C17:0	0.7 ± 0.1	1.9	1.0	1.2	0.8 ± 0.2	0.7 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
C18:0	9.5 ± 0.8	7.7	10.7	10.4	7.5 ± 0.8	5.3 ± 0.4	4.2 ± 0.5	4.3 ± 0.5	5.2 ± 0.4	5.8 ± 0.2
C20:0	2.3 ± 0.4	0.3	0.3	0.4	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
C22:0	1.5 ± 0.3	0.3	0.1	0.3	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
C23:0	2.5 ± 0.2	0.5	n.d.	0.1	0.1 ± 0.0	n.d.	0.2 ± 0.3	0.2 ± 0.1	n.d.	n.d.
C24:0	2.0 ± 0.9	0.3	n.d.	0.3	0.3 ± 0.1	0.4 ± 0.3	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
MUFA										
C16:1*n*-7	15.3 ± 0.5	0.9	2.6	4.3	1.8 ± 0.5	2.7 ± 0.6	3.4 ± 0.1	3.1 ± 0.8	3.3 ± 0.4	2.6 ± 0.2
C18:1*n*-9	7.2 ± 0.4	3.9	12.4	10.9	5.4 ± 0.9	5.5 ± 1.8	12.2 ± 1.5	9.4 ± 1.7	11.8 ± 0.9	10.2 ± 1.0
C18:1*n*-7	7.2 ± 0.2	1.9	2.9	4.2	1.8 ± 0.5	1.9 ± 0.3	1.5 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
C20:1	0.4 ± 0.1	0.3	0.3	0.4	0.3 ± 0.1	0.4 ± 0.1	1.2 ± 0.2	2.1 ± 0.5	1.0 ± 0.2	0.2 ± 0.0
C22:1	4.0 ± 1.1	6.4	0.2	0.5	0.3 ± 0.0	0.6 ± 0.2	0.9 ± 0.2	2.8 ± 0.7	0.5 ± 0.2	0.2 ± 0.1
C24:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PUFA										
C20:2	n.d.	0.2	0.6	0.4	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
C22:2	n.d.	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:2*n*-6	2.5 ± 0.0	1.1	2.5	1.7	1.2 ± 0.2	0.7 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.5 ± 0.1
C20:3*n*-6	n.d.	n.d.	1.5	0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C20:4*n*-6	n.d.	0.9	5.9	3.8	4.7 ± 1.3	3.2 ± 0.2	2.1 ± 0.8	1.7 ± 0.4	2.5 ± 0.5	3.6 ± 0.0
C22:4*n*-6	n.d.	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:5*n*-6	1.1 ± 0.2	7.0	2.0	1.2	3.0 ± 0.7	1.4 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	1.4 ± 0.2
C18:3*n*-3	2.2 ± 0.5	1.0	0.4	1.3	0.6 ± 0.2	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
C18:4*n*-3	2.0 ± 0.1	0.4	n.d.	0.3	0.3 ± 0.1	0.3 ± 0.1	1.3 ± 0.5	0.5 ± 0.1	0.5 ± 0.2	0.2 ± 0.0
C20:3*n*-3	n.d.	0.1	0.2	0.3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C20:5*n*-3	6.0 ± 0.1	16.0	4.3	17.7	11.4 ± 1.6	10.8 ± 1.1	11.5 ± 1.3	10.7 ± 1.3	11.3 ± 0.1	10.4 ± 0.1
C22:5*n*-3	0.2 ± 0.0	1.1	2.3	0.5	1.3 ± 0.4	1.5 ± 0.2	1.2 ± 0.4	1.1 ± 0.2	1.4 ± 0.1	2.0 ± 0.0
C22:6*n*3	2.7 ± 0.4	30.8	22.6	16.8	30.8 ± 4.6	39.6 ± 1.4	33.7 ± 1.1	36.7 ± 2.8	31.8 ± 1.4	34.9 ± 1.3
SFA	49.4 ± 2.5	27.5	39.2	35.6	36.5 ± 1.5	30.5 ± 0.8	28.8 ± 0.5	28.9 ± 0.4	32.1 ± 0.1	31.8 ± 0.3
MUFA	34.0 ± 1.2	13.3	18.4	20.3	9.5 ± 1.6	13.2 ± 3.7	19.2 ± 1.5	17.7 ± 1.9	18.2 ± 1.7	14.4 ± 0.9
PUFA	16.6 ± 1.3	59.2	42.4	44.1	54.0 ± 2.9	56.3 ± 3.6	52.0 ± 1.4	53.4 ± 1.5	49.7 ± 1.8	53.7 ± 1.1
TFA	0.2 ± 0.1	16.8	46.1	12.6	9.7 ± 0.4	16.4 ± 1.5	14.5 ± 2.3	15.2 ± 0.6	13.4 ± 0.5	12.8 ± 0.3
n-3/*n*-6	3.5 ± 0.1	5.23	2.51	5.47	5.2 ± 1.6	9.8 ± 0.4	13.5 ± 3.2	16.6 ± 2.8	10.9 ± 1.1	8.5 ± 0.1

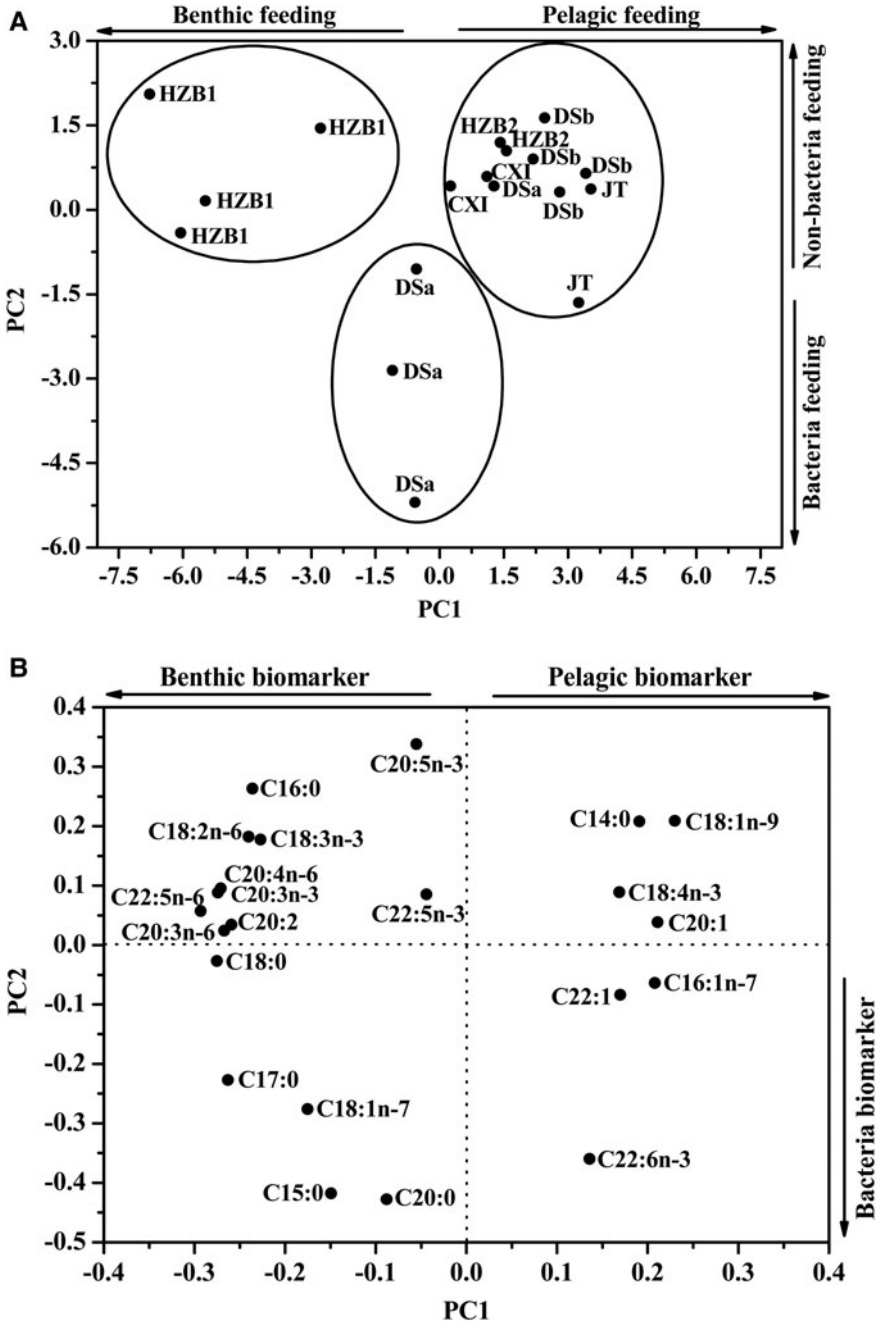

Fig. 3. Principal component analysis (PCA) of fatty acids in *Coilia mystus* (PC1: 48.9%, PC2: 14.1% of the total variance): (A) PCA scores plot showing separation among prey groups; (B) PCA factor loadings showing individual fatty acids contributing to the separation among prey groups. Those fatty acids with highest factor loadings have the greatest influence on discrimination among prey items.

based on the PCA scores of the samples from various stations, the samples were separated into three groups: M1-HZB1, M2-DSa, and a group composed of M3-DSb, M3-JT, M4-HZB2, M4-CXI. ANOSIM analysis was performed on the fatty acid compositions of *C. mystus* samples from the three groups. The results ($R = 0.683$, $P = 0.001$) of the ANOSIM suggested that the difference among the three groups was significant.

The PCA variable loading plot showed that the fatty acids determined the positions of *C. mystus* on the score plot (Figure 3B). C18:2*n*-6, C18:3*n*-3, C20:4*n*-6, C16:0, C17:0, C20:3*n*-6 and C18:1*n*-7 were on the negative side of the PC1 axis, indicating the contribution of benthic detritus biomarkers to the variance (Stowasser *et al.*, 2009a, b). However, C18:1*n*-9, C18:4*n*-3, C20:1, C22:1, C16:1*n*-7 and C22:6*n*-3 were on the positive side of the PC1 axis, indicating the contribution of pelagic zooplankton biomarkers to the variance (Kattner & Hagen, 2008). C18:1*n*-7, C15:0 and C17:0 were on the negative side of the PC2 axis, indicating the contribution of bacteria (Parrish *et al.*, 2000; Stowasser *et al.*, 2009a, b). SIMPER analysis showed that C18:1*n*-9, C22:6*n*-3, C20:5*n*-3, C20:4*n*-6, C16:0, C16:1*n*-7, C18:1*n*-7, C18:2*n*-6, C20:1, C22:1 and C18:0 contributed the most to the dissimilarity among the groups.

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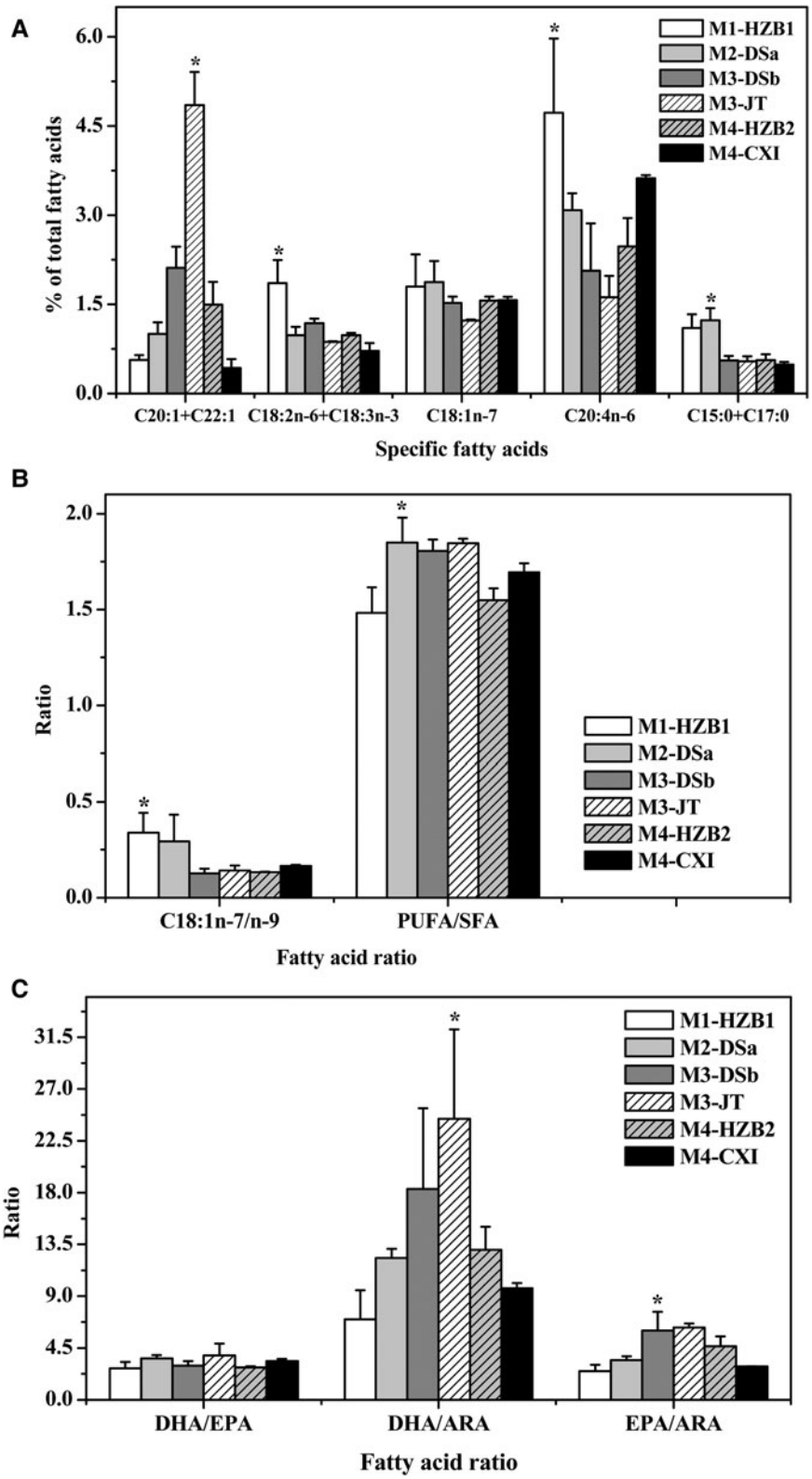

Fig. 4. Station-related changes in the fatty acid biomarkers of *Coilia mystus*: (A) specific fatty acid concentrations; (B) C18:1*n*-7 and PUFA/SFA ratios; (C) PUFA ratios. Data are shown as the average ± standard deviation. '*' indicates that the difference in the specific fatty acids of individuals in different stations was significant.

Furthermore, several specific fatty acids were chosen according to the results of the PCA and SIMPER to analyse the variations in the fatty acid composition of *C. mystus* during their migration (Figure 4). Significance of difference was labelled in the figure. The C20:1 + C22:1 concentration was high in *C. mystus* at the M3-DSb, M3-JT and M4-HZB2 samples but low in M1-HZB1, M2-DSa, and M4-CXI (Figure 4A). The change in C18:1*n*-7 was consistent with that of C20:4*n*-6, which was high in M1-HZB1 and M2-DSa, then decreased in M3-DSb, M3-JT, but increased

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in M4-HZB2, M4-CXI (Figure 4A). The C18:2*n*-6 + C18:3*n*-3 concentration was as high as 2% of total fatty acids in M1-HZB1, but only approximately 1% in other migration stages (Figure 4A). C15:0 + C17:0 and the C18:1*n*-7/*n*-9 ratios were slightly higher in M1-HZB1 and M2-DSa than in the other migration stages (Figure 4A, B).

Relationship between the biomarkers and body length of *C. mystus*

For total *C. mystus* samples, both $\delta^{13}C$ and $\delta^{15}N$ were positively correlated with the body length ($\delta^{13}C$ $r = 0.648$, $P = 0.003$, $\delta^{15}N$ $r = 0.629$, $P = 0.004$). Linear regression results showed that the body length explained 41.5% and 40.1% variation of $\delta^{13}C$ and $\delta^{15}N$ in *C. mystus*, respectively (Figure 5). There was no significant correlation between the specific fatty acids and the body length for the *C. mystus* total samples ($P > 0.05$). If only those collected from M3-DSb were considered, $\delta^{13}C$ was still positively correlated with the body length ($r = 0.881$, $P = 0.004$), but $\delta^{15}N$ was not significantly correlated with the body length ($P > 0.05$). There also was no significant difference in the C20:1 + C22:1 and C20:4*n*-6 concentrations and C18:1*n*-7/*n*-9 ratio among the four groups of individuals in M3-DSb (ANOVA, $P > 0.05$).

DISCUSSION

Potential dietary influence on the stable isotopes of *C. mystus*

$\delta^{13}C$ and $\delta^{15}N$ values are often used as indicators of the carbon source and trophic level, respectively, of organisms within the food web (Gu *et al.*, 1996; Overman & Parrish, 2001; Post, 2002). In the present study, both $\delta^{13}C$ and $\delta^{15}N$ values of *C. mystus* were enriched during the migration. Generally, the changes in the $\delta^{13}C$ and $\delta^{15}N$ values of the fish may suggest variation in the diet source and trophic level. But influences from the factors other than the diet source on the $\delta^{13}C$ and $\delta^{15}N$ values should be born in mind, especially for the migration species undergoing habitat shift. First, large individuals tend to accumulate heavy isotopes during the metabolic process, so the metabolic effects associated with life stage cannot be totally ignored when performing stable isotope analysis (Malej *et al.*, 1993; Overman & Parrish, 2001). This accumulation increases the risk of a bias in prey source identification when long term variation is studied. Correlation between stable isotopes and body length has been identified in *C. mystus*. Even the individuals in the same migration stage exhibited enriched $\delta^{13}C$ with increasing body length. The body lengths of individuals in stage M4 were higher than those in stage M1. Thus, it is possible that the increases in the average $\delta^{13}C$ and $\delta^{15}N$ values of *C. mystus* during migration are partly attributed to the metabolic accumulation related to the body length. But in view of the fact that the inter-individual $\delta^{13}C$ variation for animals having a similar food source usually does not exceed 2‰ (Bardonnet & Riera, 2005), it is very likely that the dietary influence contributed to the high increase (6.1‰) in *C. mystus* to a certain degree.

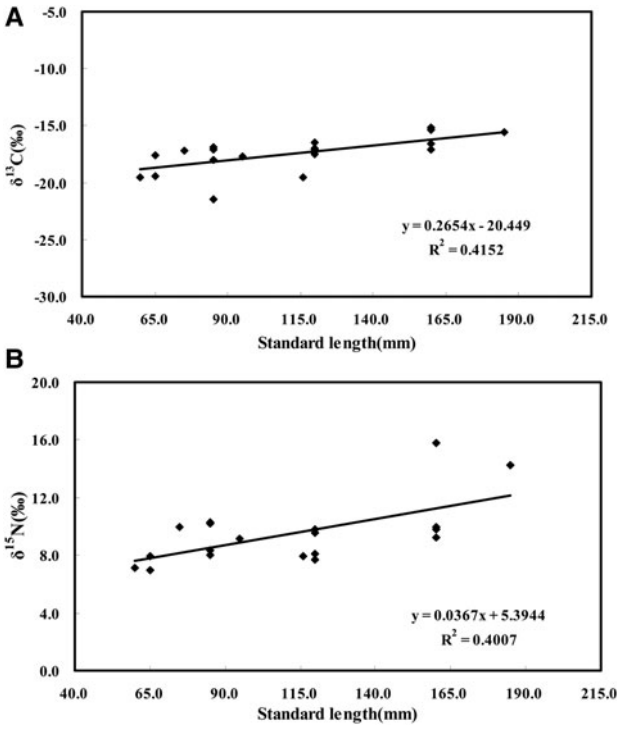

Fig. 5. Relationships between stable isotopes and body length of *Coilia mystus*: (A) $\delta^{13}C$; (B) $\delta^{15}N$.

Second, it has been shown that both overwintering and spawning could lead to increased $\delta^{13}C$ and $\delta^{15}N$ values due to the reduction in lipid content caused by the usage of lipid as fuel during fasting or migration, and the increase in $\delta^{13}C$ was normally higher than that of $\delta^{15}N$ (Ciancio *et al.*, 2008). Because the lipid content was positively related to the TFA, it can be inferred that the lipid content of *C. mystus* increased from M1 to M4 according to the corresponding TFA content. The increase in lipid content should lead to depleted $\delta^{13}C$ values because lipid is depleted in $\delta^{13}C$ (Bardonnet & Riera, 2005). However, the average $\delta^{13}C$ and $\delta^{15}N$ values of *C. mystus* increased 6.1 and 8.9‰ from M1 to M4, respectively. So, influences of overwintering and spawning on the $\delta^{13}C$ and $\delta^{15}N$ values were not observed.

Third, human impact is a potential contributor to the high $\delta^{15}N$ value in coastal regions. The biota in the aquatic ecosystem with high anthropogenic nutrient inputs tends to have highly elevated $\delta^{15}N$ value (Steffy & Kilham, 2004; Mercado-Silva *et al.*, 2009; Herbeck, 2011). In a previous study, the average $\delta^{15}N$ value of organisms in highly human-impacted region was 10‰ enriched than those in less impacted system in Mexico (Mercado-Silva *et al.*, 2009). Samples at Stations CXI and HZB2 were all at stage M4, the effect from the metabolic accumulation is supposed to be similar. Thus, a factor other than the physiological accumulation may lead to the significantly higher $\delta^{15}N$ values of those in M4-CXI. In addition, the average $\delta^{15}N$ value of seston collected in Station CXI was as high as 6.9 $\pm$ 0.8‰, even higher than that of *A. chinensis* collected from Hangzhou Bay. The Station CXI is near the large city of Shanghai and may be extensively influenced by anthropogenic run-off. So, it can be suggested that anthropogenic run-off may contribute to the high $\delta^{15}N$ values in *C. mystus* at M4-CXI.

Although there are simultaneous influences from metabolic accumulation and anthropogenic effect, the dietary influence on the $\delta^{13}C$ and $\delta^{15}N$ pattern of *C. mystus* can be indicated. The habitat of the migration species is variable

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during their life stages. Considering the possible difference in the baseline of the food web, only the trophic level (TL) of *C. mystus* in the stage M1 was calculated with *A. Chinensis* collected in HZB1 as the baseline. Individuals in stage M1 were secondary consumers, roughly at the same TL as the planktivore fish *Engraulis japonicus*, and both preyed predominantly on zooplankton (Wang, 2008). Even if the extraordinarily high values of M4-CXI were supposed due to the anthropogenic effect, the individuals in M3-DSb and M4-HZB2 also exhibited more enriched $\delta^{15}N$ values than those of stage M1. The enrichment in $\delta^{15}N$ values has been attributed to changes in prey type and the size of prey (Jennings *et al.*, 2002; Xu *et al.*, 2007; Stowasser *et al.*, 2009a). So the increase in the $\delta^{15}N$ values of *C. mystus* during migration may suggest that the diet compositions of *C. mystus* at various migration stages may be different, and this species may prey at different TLs when it is at different life stages. Previous gut content analyses of *C. mystus* indicate that although zooplankton account for over 60% of the *C. mystus* diet, items such as small shrimp (e.g. *Leptochela gracilis*) and small fish (e.g. goby) in higher TLs than zooplankton are also included (Zhou *et al.*, 2004; Zhuang *et al.*, 2010). Diet shift of *C. mystus* was also documented in previous studies. It was determined that *C. mystus* smaller than 60 mm feed primarily on copepods, cladocerans and amphipods. Those individuals 60–150 mm feed predominantly on polychaetes, decapods, chaetognathans and small fish. Decapods, mysidaceans, and small fish were the dominant diet items of individuals larger than 150 mm (Yuan & Qin, 1984; Ni *et al.*, 1999). Variation in the TL of a species has been found in many fish (Zhang, 2004; Xu *et al.*, 2007; Stowasser *et al.*, 2009a; Wan *et al.*, 2010). For instance, the TL of the large lake anchovy (>130 mm) was 0.6 higher than that of small individuals (<130 mm). The difference between individuals >180 and <180 mm was even as high as one level for *Coryphaenoides armatus* (Stowasser *et al.*, 2009a).

Potential dietary influence on fatty acid composition of *C. mystus*

The variation in the $\delta^{13}C$ and $\delta^{15}N$ patterns of *C. mystus* during migration suggested that their diet sources may vary during different life stages. However, due to the potential influence of metabolic accumulation and the environment change, the dietary influence on the stable isotopes requires further confirmation with fatty acid composition. The biosynthesis and storage of the fatty acids in organisms usually follow several regular patterns, making them useful tracers of diet source (Iverson, 2008). Both specific fatty acid and overall fatty acid signature can be used as tracers (Budge *et al.*, 2006). The correlation between body size and fatty acid composition was not supported by the correlation analysis. Thus, it can be supposed that variations in the fatty acid composition are mainly due to the shift of diet source.

The significant difference in the fatty acid compositions of *C. mystus* in stage M1, stage M2 and stages M3, M4 may confirm that variation in diet composition occurred during migration. The dietary influence can also be reflected in the variation in the specific fatty acids. Generally, high C18:1*n*-9 content and low C18:1*n*-7/*n*-9 ratio may indicate carnivorous feeding behaviour of zooplankton (Petursdottir *et al.*, 2008; Brett *et al.*, 2009). In addition, a high PUFA/SFA ratio also indicates carnivorous behaviour of zooplankton. Herbivorous zooplankton exhibits a comparatively lower PUFA/SFA ratio because seston usually contains a high SFA content and is depleted in PUFA (Rossi *et al.*, 2008). And there is a general trend that PUFA accumulates from low (phytoplankton and detritus) to high TLs (anchovy larvae) (Rossi *et al.*, 2006). In light of the limited ability of the marine fish to biosynthesize the fatty acids *de novo* (Iverson, 2008), it can be supposed that the fatty acid pattern of the zooplankton can be reflected in the fatty acid pattern of their predator (Rossi *et al.*, 2006). *Coilia mystus* in stages M1 and M2 preyed on lower TL than those in other stages, as indicated by the higher C18:1*n*-7/*n*-9 ratios compared to other stages, which was consistent with the result that the $\delta^{15}N$ values of *C. mystus* in stages M1 and M2 were lower than those in stages M3 and M4. Low PUFA/SFA ratios in stage M1 samples may also suggest the low carnivorous level of *C. mystus* in that stage. The PUFA/SFA ratios of *C. mystus* in stage M2 were elevated, possibly because high PUFA concentrations were needed for *C. mystus* to resist low temperatures in March, considering their physiological function related to the membrane fluidity at low temperatures (Hall *et al.*, 2002). Our results also showed that the C18:1*n*-7/*n*-9 ratio was a more reliable biomarker of carnivorous activity than the PUFA/SFA ratio.

More detailed information on the diet items can be suggested by several specific fatty acids. C20:1 + C22:1 has been used as biomarker of copepods in food web study (Kattner & Hagen, 2008; Rossi *et al.*, 2008). High level of C20:1 + C22:1 concentration in fish generally indicates diet containing copepods (Graeve *et al.*, 2008; Stowasser *et al.*, 2009a, b). Copepods were important diet items for stage M3 *C. mystus* collected from DSb and JT as indicated by the high C20:1 + C22:1 concentration in their muscle. The gut content analysis of *C. mystus* collected from HZB2 and CXI found that copepods and mysidaceans were the dominant diet items for these fish (Liu & Xu, 2011). In addition to the pelagic copepods mentioned above, the contribution of benthic food items can also be confirmed by the specific fatty acids found in *C. mystus*. C18:2*n*-6 and C18:3*n*-3 have been used as indicators of the contribution of terrestrial organic matter because they are typical fatty acids in freshwater and terrestrial primary producers (Parrish *et al.*, 2000; Iverson, 2008). However, some marine macroalgae also contain high levels of C18:2*n*-6 + C18:3*n*-3 and C20:4*n*-6 (Li *et al.*, 2002; Koussoroplis *et al.*, 2011). Whether from terrestrial or marine primary producers, the most likely mechanism for their entrance into the food web is in the form of detritus, because few organisms can graze on macrophytes directly. The presence of C18:2*n*-6 + C18:3*n*-3 in the seston samples might reflect a significant contribution of detritus in the phytoplankton fraction (Graeve *et al.*, 2002). C18:1*n*-7 can be biosynthesized by bacteria and diatoms, and also in high concentration in some macroalgae species (Volkman *et al.*, 1998; Li *et al.*, 2002). It has been used as a biomarker of bacteria (Alfaro, 2008). C20:4*n*-6 is found in high concentrations in benthic organisms such as amphipods, echinoderms, crabs and deep-sea polychaetes (Copeman & Parrish, 2003; Hall *et al.*, 2006; Maazouzi *et al.*, 2007; Würzberg *et al.*, 2011). Generally, C20:4*n*-6 is found in low concentrations in most marine fish, but fish that prey on exclusively benthic foods (e.g. benthic shrimps) tend to contain high C20:4*n*-6 concentrations (Stowasser *et al.*,

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2009a, b, Wan *et al.*, 2010; Koussoroplis *et al.*, 2011). Thus, C18:1*n*-7 and C20:4*n*-6 can be used as indicators of benthic food sources within the food chain (Stowasser *et al.*, 2009a, b). In this study, the C20:4*n*-6 concentration was high in fish larvae and *A. chinensis*. The high concentrations of C18:1*n*-7 and C20:4*n*-6 in *C. mystus* collected from HZB1 indicated the contribution of benthic-origin organic matter to their diet, which may be transferred through grazing on benthic organisms directly or through preying on those who consume benthic organisms (e.g. mysidaceans, fish larvae and *A. chinensis*). Benthic food has been found in the diet of *C. mystus* in previous studies (Luo *et al.*, 1997; Li *et al.*, 2009). According to our results, the concentrations of benthic biomarkers and pelagic biomarkers in *C. mystus* in different stages were changing. Therefore, it can be suggested that the ratio of pelagic to benthic food sources is variable in *C. mystus* during migration.

The higher C18:2*n*-6 + C18:3*n*-3, C18:1*n*-7, C20:4*n*-6 and C15:0 + C17:0 concentrations and lower C20:1 + C22:1 concentration in M1 *C. mystus* suggest that the detritus-derived benthic food chain contributed more to their diet than copepods. Individuals in stage M2 were also characterized by high C18:1*n*-7, C20:4*n*-6 and C15:0 + C17:0 concentrations and a lower C20:1 + C22:1 concentration. It appears that the benthic food web, especially bacteria, contributed more than the pelagic food web to the diet composition of stage M2 *C. mystus*. However, compared with individuals from stage M1, the C18:2*n*-6 + C18:3*n*-3 concentration was lower in stage M2 *C. mystus*. Using C18:2*n*-6 + C18:3*n*-3 as an indicator of the contribution of terrestrial matter, it appears that terrestrial organic matter contributed more to stage M1 *C. mystus* than to those in stage M2. This difference may due to the difference in the geographic locations of the stations where they were collected. The station where *C. mystus* in stage M1 were collected was closer to the land, where terrestrial matter was more dominant, compared to the station where *C. mystus* in stage M2 were collected. Conversely, stage M3 *C. mystus* and stage M4 individuals collected at the HZB2 station contained high concentrations of the copepod biomarker C20:1 + C22:1 and low levels of benthic biomarkers (i.e. C18:2*n*-6 + C18:3*n*-3, C18:1*n*-7, C15:0 + C17:0, C20:4*n*-6), suggesting that pelagic copepods dominated the *C. mystus* diet over foods from benthic sources. Compared with stage M3 *C. mystus*, the C20:1 + C22:1 concentration of stage M4 individuals decreased and concentrations of C18:1*n*-7 and C20:4*n*-6 increased, whereas the concentration of C15:0 + C17:0 did not change. This variation in the fatty acid biomarkers suggests that the dietary contribution of copepods decreased in stage M4 and the contribution of benthic food increased, but that from bacteria remained the same. This also indicates that the benthic food of M4 stage *C. mystus* was at a higher trophic level than that of stage M1 or M2 *C. mystus*.

Marine organisms often contain high levels of *n*-3 PUFA, and terrestrial organisms often contain high *n*-6 PUFA. Thus, the *n*-3/*n*-6 PUFA ratio can be used as an indicator of the amount of marine-derived vs terrestrial-derived food in an organism's diet (Hebert *et al.*, 2006, Ahlgren *et al.*, 2009). The *n*-3/*n*-6 ratio was lower in stage M1 *C. mystus* and very high in stage M3 individuals. This finding is consistent with the above conclusion that terrestrial-derived organic matter contributed more to the diet of stage M1 *C. mystus,* and marine-derived organic matter was the dominant food source for individuals in stage M3. The *n*-3/*n*-6 ratio of stage M4 *C. mystus* decreased compared to M3, which indicates that the proportion of terrestrial-derived food sources in their diet increased again.

The trophic strategy of *C. mystus* during migration as indicated by the stable isotopes and fatty acid composition can be described as follows: *C. mystus* in stages M1 and M2 prey on lower TL compared with those in stages M3 and M4. When they are in stage M1, items derived from benthic and terrestrial based food webs dominate their diet. When they migrate to the Zhoushan Islands to pass the winter, benthic organisms remain their dominant food source. However, in May the contribution of pelagic copepods to the diet of stage M3 *C. mystus* dramatically increases, and copepods become their dominant dietary source. For *C. mystus* in stage M4, the contribution of copepods decreases and the contributions of benthic food sources increase again. Notably, the benthic food source for stage M4 individuals is in a higher TL than those in stages M1 and M2. Bacteria contribute more to the diet when individuals are in stages M1 and M2 than when they are in stages M3 and M4.

Seasonal and spatial variation in the diet composition of fish is a common phenomenon (Letourneur *et al.*, 1997; Mandima, 2000; Schafer *et al.*, 2002; Xue *et al.*, 2004; Zhang *et al.*, 2008; Bacha & Amara, 2009; Stowasser *et al.*, 2009a; Xue *et al.*, 2010). Diet variation may be closely related to the seasonal or spatial availability of the dominant prey types (Mandima, 2000; Zhang *et al.*, 2008). Zooplankton in the Changjiang Estuary exhibited seasonal and spatial variation in both abundance and species dominance (Zhu, 1988; Xu *et al.*, 1995; Xu & Shen, 2005; Zhu *et al.*, 2011). The abundance of zooplankton was very low in March but high in May (Zhu, 1988; Xu & Shen, 2005). This may explain why the diet compositions of M2 and M3 *C. mystus*, which were both collected from the Daishan Islands, were different. In March, when there were not sufficient copepods for food, organic matter originating from the benthic detritus food web became the main dietary source for M2 *C. mystus*. However, M3 individuals could prey predominantly on copepods (e.g. *Calanus sinicus*) because the abundance of zooplankton was very high in May. Both Stations CXI and HZB1 are located in regions where zooplankton abundance is low (Zhu, 1988; Xu & Shen, 2005; Ji & Ye, 2006; Zhu *et al.*, 2011). However, Stations DS, JT and HZB2 were close to the Zhoushan Islands, which are characterized by high zooplankton abundance and high fishery catch (Zhu, 1988; Xu & Shen, 2005; Ji & Ye, 2006; Zhu *et al.*, 2011). The high availability of zooplankton in the waters around the Zhoushan Islands may result in the high contribution of zooplankton to the diets of M3 and M4-HZB2 *C. mystus*. Therefore, the dietary influence on the fatty acid composition of *C. mystus* may be related to the food availability in these different regions and seasons.

CONCLUSION

Though it may be covered by other factors, such as metabolism accumulation and environment shift, the influence of dietary source on stable isotopes of *C. mystus* during migration can be confirmed by the fatty acid composition information. According to the variation in the biomarkers, individuals at different migration stages seemed to have distinct diet sources. The pelagic (e.g. copepods) and benthic (e.g.

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mysidaceans) food sources alternatively dominated the diet items of *C. mystus* during migration, as suggested by the specific fatty acids. This study demonstrated the usefulness and feasibility of stable isotopes and fatty acid composition in determining the feeding habits of migratory fish in an estuary. Fatty acid composition was more sensitive in reflecting the dietary variation and can provide more detailed dietary information than bulk stable carbon and nitrogen isotopes.

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