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Factors affecting lipid and fatty acid composition of *Calanus sinicus* in the Yellow Sea and the East China Sea in spring*

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Abstract The factors affecting lipid and fatty acid composition of copepod *Calanus sinicus* in the Yellow Sea (YS) and the East China Sea (ECS) were examined in this study. In spring, there were significant differences between these two regions for both environmental conditions and food availability. Such regional difference significantly influenced the lipid and fatty acid profiles of *C. sinicus*. Our results show that *C. sinicus* has a higher lipids content in ECS, especially for wax ester and triglyceride lipids, indicating a more active and efficient predation. According to BIO-ENV analysis, the variation of lipids profiles may be influenced majorly by water temperature. Moreover, the fatty acids (FAs) profiles of *C. sinicus* were also different between YS and ECS, especially in the four major contributors, C22:1ω11, eicosapentaenoic acid (EPA), docosahexenoic acid (DHA), and C20:1ω9. The considerable amounts of self-biosynthesized FAs of herbivorous copepod (C22:1ω11 and C20:1ω9) and low DHA/EPA ratio may indicate that *C. sinicus* in ECS feed mainly on phytoplankton comparing to those in YS. The fatty acid profiles of *C. sinicus* were affected by the differences in food availability.

Keyword: *Calanus sinicus*; lipid storage; wax ester; fatty acid composition; Yellow Sea and East China Sea

1 INTRODUCTION

Many *Calanus* species, especially those from Polar Regions, can store a large amount of triacylglycerols or wax esters in oil sacs or oil droplets (Lee et al., 2006). The stored lipids play important roles in the life history of marine *Calanus* in reproduction, ontogeny, diapause, and coping with food scarcity, as shown by studies in various oceanic regions (Rey-Rassat et al., 2002; Mayor et al., 2009; Pond and Tarling, 2011; Clark et al., 2012). Moreover, the transport and metabolism of the carbon-rich lipids by diapausing copepods such as *Calanus finmarchicus* provide another efficient way of sequestering carbon into the ocean floor (Jónasdóttir et al., 2015).

The lipid accumulation process is influenced by both intrinsic (lipid metabolism genes) and external environmental (such as temperature, food quality and quantity) factors (Koussoroplis et al., 2014; Zhou et al., 2016; Zhou and Sun, 2017). However, existing knowledge about how these factors affect the lipid accumulation process in *Calanus* is still limited. Zhou and Sun (2017) argued that low temperature especially diurnal temperature differences might promote lipid

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accumulation in *Calanus sinicus* by reducing the energy output at colder temperatures and extending the lipid accumulation duration. However, this was based on the result of laboratory work rather than field investigation. Additionally, lipid accumulation is also closely related to food availability and food composition. According to previous studies, lipid accumulation is higher in copepods cultured in high concentration diets (Hakanson, 1984; Escribano and McLaren, 1992; Hygum et al., 2000; Rey-Rassat et al., 2002). However, in the field, *C. sinicus* at copepodite stage V (CV) develops larger oil sacs in the Yellow Sea Cold Water Mass where the food is deficit (Wang et al., 2009). This indicates that other factors, apart from food availability, may also affect lipid accumulation in *Calanus*. In addition to food quantity, prey type can also influence lipid accumulation (Hygum et al., 2000). Pepin (2011) found that higher lipid accumulation was associated with *C. finmarchicus* at CV stage from a diatom dominated region and lower lipid accumulation was associated with those from dinoflagellates and prymnesiophytes dominated area. However, in the laboratory, *C. sinicus* showed a significantly higher level of total lipid content in *Prorocentrum micans* (dinoflagellate) diet than in *Skeletonema costatum* (diatom) diet (Liu et al., 2011).

Calanus sinicus is the dominant meso-zooplankton species in the Northwest Pacific Ocean, contributing 73%–99% of copepod biomass in the Yellow Sea (YS) (Sun et al., 2010), and 4%–76% in the East China Sea (Xu and Chen, 2007). Due to its high abundance and wide distribution, *C. sinicus* plays a key role in energy transfer from phytoplankton to higher trophic levels. Our previous work has studied the role of lipids during the over-summering period of *C. sinicus* in the YS (Wang et al., 2017). In this work, we collected *C. sinicus* from the East China Sea (ECS) and the YS to explore whether the lipid accumulation traits of *C. sinicus* is affected by different environmental conditions.

2 MATERIAL AND METHOD

2.1 Sample collection and environmental conditions

The study was carried out in the YS and ECS during the cruises in spring (R/V *Kexue 3*, April 6–25, 2011). Samples and environmental data were collected in 3 stations in southern YS and 4 stations in the ECS (Fig.1).

Temperature and salinity data were collected using a Seabird Electronics 25 CTD and only the data of 5-m layer is shown in this study. Chlorophyll-*a* (Chl-*a*) values were determined by collecting 1 L of water from the maximum Chl-*a* stratum using a Seabird Electronics 25 CTD. Water samples were filtered onto 0.45-μm acetate fiber filters, extracted in 90% acetone for 24 h, and Chl-*a* concentrations were determined using a Turner Design Fluorometer (7200).

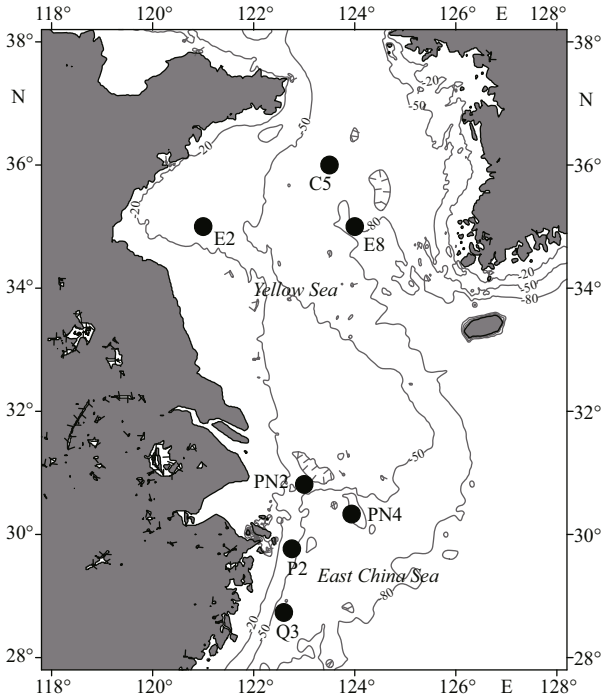

Fig.1 The sampling stations in the Yellow Sea and the East China Sea

Phytoplankton samples were collected using a plankton net (70-μm mesh size, mouth opening 0.1 m^2, with a flow-meter inside), which was hauled vertically from 4 m above the seabed to the surface. The samples were preserved in 5% formalin seawater solution, and phytoplankton was analyzed microscopically. Ciliate data of this cruise from Ding and Xu (2012) was used for data analysis in the current study.

Zooplankton samples were got by another kind of plankton net (500-μm mesh size, mouth opening 0.5 m^2, with a flow-meter inside), which was also hauled vertically from 4 m above the seabed to the surface. Samples were preserved in 5% formalin seawater solution. *C. sinicus* individuals were counted under a dissecting microscope (Nikon SMZ645, Japan) from copepodite stage III (CIII) to adults (males and females). For lipid and fatty acid analysis, another vertical haul was conducted using the plankton net. *C. sinicus* individuals were picked

out according to different developmental stages. However, the CIII stage and male individuals were not picked out because of their small body size and/or little abundance. Then the picked samples were rinsed with Mill-Q (Sartorius H2Opro, Germany) waters and stored in liquid nitrogen until lipid analysis.

2.2 Lipid analysis

Lipids were extracted from *C. sinicus* following the extraction procedures of Folch et al. (1957) and Parrish (1999). Briefly, samples were lyophilized at -45 °C for 48 h. Total lipids were extracted using 3-mL chloroform:methanol (2:1, v:v) at -20 °C for 16 h. The lower chloroform phase containing the lipid extracts was transferred to a glass vial. After the addition of 0.75-mL KCl (0.88%, w:v), the vials were whirl-mixed and centrifuged (400×*g*, 2 min). The lower organic phase was transferred to a pre-combusted glass tube. Lipid extract was evaporated under high purity nitrogen and total lipid content was determined gravimetrically.

Wax ester, polar lipids, and triglyceride were separated by chromatography and analyzed in duplicate using an Iatroscan® MK-6 (Mitsubishi Chemical Medience, Japan) with a flame ionization detector (Hagen, 2000). Total lipid samples were transferred to Chromarods-SIII (Mitsubishi Chemical Medience, Japan) using a microcapillary pipette (1 μL). These rods were developed twice, initially using hexane:benzene (1:1, v:v) and then using benzene:chloroform:acetic acid (50:20:0.7, v:v:v). The twice-developed Chromarods were then scanned on the machine at a speed of 30 s per rod and a hydrogen flow rate of 160 mL/min. Following Ohman (1997), wax ester standards for calibration were purified from *C. sinicus* at CV stage (~500 inds.) collected from the field in August. Commercial standards were used for quantitative analysis: triglyceride mix for triacylglycerol and phosphotidylcholine for polar lipids.

2.3 Fatty acid analysis

Lipid classes were separated via column chromatography following Ohman (1997). Wax esters were obtained by eluting 1% diethyl ether in hexane and the eluent was collected and evaporated under high purity nitrogen. The wax ester extracts were then trans-esterified in 3-mL methylation reagent (methanol:sulfuric acid=99:1, v:v) at 50 °C for 16 h (Wang et al., 2017). After the addition of 2-mL Milli-Q water and 3-mL hexane:diethyl ether (1:1, v:v), samples were centrifuged (1 000×*g*, 10 min) for stratification. The upper layer was transferred to a pre-combusted vial and re-washed using 1-mL 2% (w:v) $NaHCO_3$. Organic layer was separated and purified in thin layer chromatography (hexane:benzene=1:1, v:v) and fatty acid methyl esters were evaporated under high purity nitrogen. Analysis of fatty acids was carried out using an Agilent 7890A Gas Chromatography instrument equipped with a DB-FFAP capillary column (30-m length, 0.25-mm inner diameter, and 0.25-μm film thickness). The temperature programming was as follows: 150 °C for 1 min, heat at 3 °C/min to 220 °C, then hold it for 33 min. The temperatures of the injector and the detector were maintained at 220 °C and 280 °C, respectively.

2.4 Data analysis

PRIMER software (Plymouth, UK) version 6 (Clarke and Ainsworth, 1993) and R software version 3.2.3 were used for data analyses.

The abundance and population compositional data were subjected to q-type cluster analysis based on the Bray-Curtis dissimilarity index and group average linkage classification (Field et al., 1982). Based on the results of cluster analysis, nonparametric tests were used to analyze differences in lipid composition between different cluster groups.

The fatty acid proportional data were analyzed using ANOSIM to test for differences between groups (cluster groups and developmental stages). Similarity Percentages **(**SIMPER**)** and Principal Component Analysis (PCA) were used to determine the main fatty acids contribution to these differences (the SIMPER test was set at 70% cumulative contribution).

The BIO-ENV procedure (PRIMER software) was used to estimate which set of environmental variables (temperature, salinity, Chl *a*, phytoplankton abundance, and ciliates) best explained the differences in lipid and fatty acid profiles of *C. sinicus* from the YS and the ECS. All the data used in this analysis were fourth root transformed. BIO-ENV analysis is based on determining the Spearman's rank correlation coefficient (qw) between biological and environmental similarity matrices. A value of qw=0 would imply no match between the two matrices, while a value of qw=1 means a perfect match (Clarke and Ainsworth, 1993).

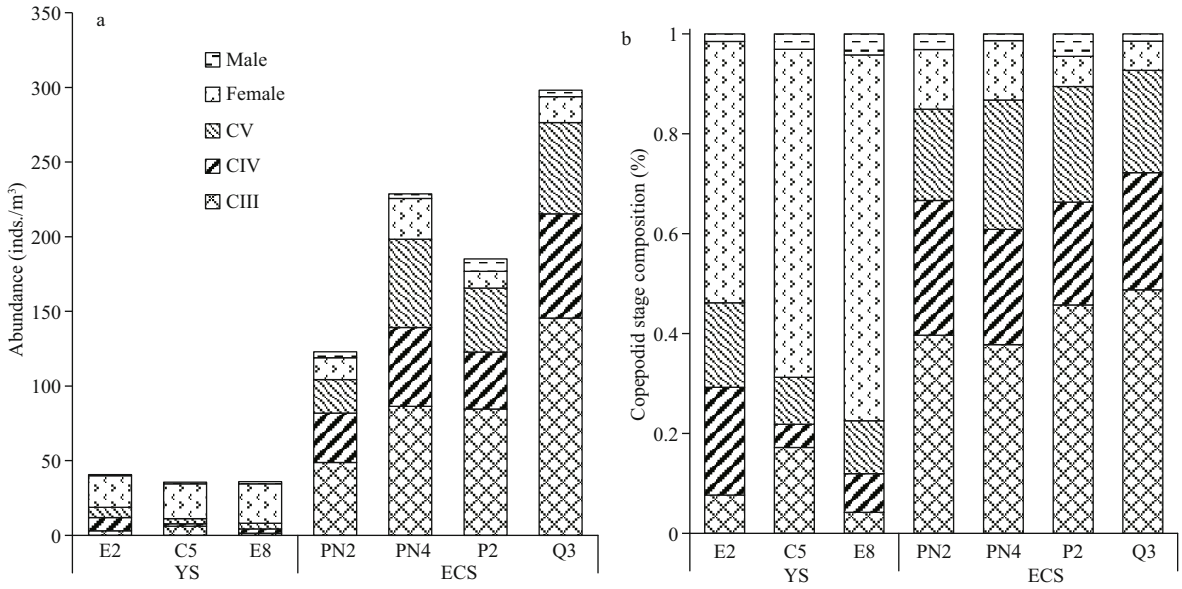

Fig.2 Abundance (a) and development stage composition (b) of *C. sinicus* at each sampling station in the Yellow Sea (YS) and East China Sea (ECS)

CIII\CIV\CV: *C. sinicus* at CIII or CIV or CV stage.

Table 1 Lipid composition of *C. sinicus* in the Yellow Sea and the East China Sea

Region	Development stage	Dry weight (μg/ind.)	Total lipid (μg/ind.)	Wax ester (μg/ind.)	Polar lipid (μg/ind.)	Triglyceride (μg/ind.)	Reference
Yellow Sea	CIV	49.0±11.3	5.3±1.9	0.6±0.5	4.4±1.3	0.1±0.2	This study (April, 2011)
	CV	157.0±44.3	18.0±0.4	2.7±0.9	14.2±1.0	0.6±0.2	
	Female	310.1±28.7	29.5±5.3	2.3±1.0	22.8±2.8	1.3±0.5	
East China Sea	CIV	70.2±7.9	14.6±3.5	6.7±3.4	6.0±0.6	1.4±0.6	
	CV	189.3±19.4	56.6±9.0	35.8±9.5	13.6±2.3	4.7±1.6	
	Female	250.2±17.4	46.1±13.2	20.6±12.6	20.2±1.7	4.0±1.5	
Yellow Sea	CV	182.8±34.5	78.8±26.0	64.2±22.8	10.2±2.2	4.0±1.8	Wang et al. (2017) & our unpublished data (June, 2011)
	Female	226.9±23.6	27.5±8.6	9.8±4.5	14.6±3.4	1.8±0.7	

CIV\CV: *C. sinicus* at CIV or CV stage.

3 RESULT

3.1 Abundance and development stage composition of *Calanus sinicus*

There were significant differences in the abundance and development stage composition of *C. sinicus* (from CIII to adult) in the YS and the ECS (Fig.2). Mean *C. sinicus* abundance was 41 inds./m^3 in the YS and 224 inds./m^3 in the ECS. *C. sinicus* in the YS were dominated by female (52%–82%), while the majority (80%–94%) of *C. sinicus* from the ECS stations was copepodites (CIII to CV).

3.2 Lipids content of *Calanus sinicus*

The dry weight of *C. sinicus* from the two regions showed a similar increasing trend from early developmental stages to the adult stage (Table 1). Similarly, increasing trends were also detected in total lipid and the three lipid classes in *C. sinicus* from CIV to the adult stage, with wax ester and polar lipid being the dominant lipid class in the ECS and the YS individuals, respectively.

In addition to polar lipid, the values of total lipid, wax ester and triacylglycerol at the same stage were significantly higher in the ECS individuals than in the YS samples. The largest regional difference was observed in wax ester at the CV stage, with a mean value of 35.8 μg/ind. in the ECS samples and 2.7 μg/ind. in the YS samples. Values of wax ester content varied from 0.6 μg/ind. in the CIV stage in YS to 35.8 μg/ind. in the CV stage in ECS (Table 1). Triglyceride levels were found to be low in both sampling regions and displayed a similar variation

Table 2 Fatty acid composition (%) of wax esters in *C. sinicus* from the Yellow Sea and the East China Sea

Fatty acid	Yellow Sea			East China Sea		
	CIV (*n*=3)	CV (*n*=3)	*F* (*n*=3)	CIV (*n*=4)	CV (*n*=4)	*F* (*n*=3)*
C14:0	1.3±1.0	2.8±2.0	1.2±0.8	0.8±0.8	2.1±1.7	2.3±3.4
C15:0	0.1±0.2	0.4±0.2	0.3±0.1	0.1±0.1	0.2±0.1	0.3±0.3
C16:0	5.2±1.8	7.7±0.6	10.8±3.9	4.0±1.3	4.7±1.4	6.2±1.4
C16:1ω7	10.4±4.5	11.6±1.6	7.6±2.6	7.8±3.2	9.6±4.3	7.8±2.7
C16:2ω4	1.6±0.7	1.3±0.1	0.7±0.6	1.2±0.5	1.6±0.6	1.4±0.7
C17:0	1.5±0.9	0.5±0.4	0.8±0.4	1.8±1.0	1.4±0.3	1.3±0.9
C16:4ω3	2.3±1.6	1.4±0.3	1.0±0.9	3.0±2.3	3.8±1.8	1.1±0.5
C18:0	1.3±0.2	1.0±0.4	1.7±0.4	1.1±0.4	1.0±0.4	1.3±0.4
C18:1ω9	2.7±0.9	4.1±0.6	4.7±3.6	1.7±0.4	1.2±0.5	3.0±0.1
C18:1ω7	0.5±0.1	0.7±0.4	1.3±0.2	0.4±0.3	0.4±0.3	0.6±0.4
C18:2ω6	1.6±0.3	2.3±0.7	3.3±3.4	0.9±0.3	0.4±0.4	1.5±0.3
C18:3ω3	1.1±0.3	1.9±0.7	2.1±2.6	0.5±0.2	0.5±0.3	1.3±0.8
C18:4ω3	6.2±1.6	8.7±1.7	5.0±4.2	6.3±1.0	5.8±1.3	5.9±3.7
C20:0	0.5±0.3	0.1±0.1	0.4±0.5	0.3±0.2	0.2±0.1	0.5±0.4
C20:1ω9	2.5±1.7	5.1±1.7	3.7±3.1	6.3±1.2	7.9±0.5	9.2±2.2
C20:1ω7	0.1±0.1	0.2±0.1	0.3±0.1	0.3±0.1	0.2±0.0	0.3±0.2
C20:2ω6	2.2±2.4	0.5±0.4	2.9±2.7	0.2±0.1	0.1±0.1	0.3±0.0
C20:3ω6	0.0±0.1	0.2±0.1	0.1±0.2	0.2±0.1	0.2±0.1	0.1±0.1
C20:4ω6	0.6±0.1	0.6±0.1	0.5±0.2	0.8±0.2	0.6±0.1	0.5±0.2
C20:4ω3	1.0±0.2	1.4±0.5	1.5±0.8	1.1±0.4	0.9±0.3	1.2±0.4
EPA	16.9±8.7	13.5±0.8	9.9±2.0	21.3±4.6	19.5±2.9	12.7±3.6
C22:1ω11	12.8±4.4	8.9±0.9	6.9±6.6	17.2±3.1	18.2±2.0	21.0±6.9
C22:1ω9	4.6±5.3	2.1±1.8	5.9±1.8	1.5±0.9	0.8±0.9	1.6±1.5
DHA	6.9±1.3	9.8±2.9	10.9±4.5	6.3±1.8	5.7±1.4	6.8±1.6

*: one of the female samples in the East China Sea was lost during the experiment processes. Mean values±standard errors.

trend with wax esters (Table 1).

In contrast with wax esters, polar lipids showed no regional differences, but did display clear differences between developmental stages (Table 1). Polar lipid values ranged from 4.4 μg/ind. in the CIV stage, to over 20.0 μg/ind. in adults.

Compared with data in June (Table 1), the wax ester content of CV did not reach the highest value in April in both regions. However, the wax ester content of female decreased during the next spring period (usually March to June in this study area).

3.3 Fatty acid composition of wax esters

The fatty acid compositions of wax esters in *C. sinicus* showed different regional characteristics between the YS and the ECS (ANOSIM, $P<0.01$, Tables 2 & 3). ANOSIM analysis was conducted to identify the differences in fatty acid compositions of wax esters. The results showed that there were significant differences in fatty acid compositions of wax esters between the YS and the ECS. However, no differences were identified among the developmental stages in the YS or the ECS (Table 3).

Table 3 Results of ANOSIM analysis based on the fatty acid information in Table 2

	P value	Global *R* stat.
a	0.03	0.19
b	0.25	0.08
c	0.00	0.42

Global *R* and *P* value of similarities among CIV, CV, and female from the Yellow Sea (a), the East China Sea (b), or among samples grouped by the Yellow Sea and the East China Sea without consideration of different development stages (c).

We identified the regional differences in fatty acid compositions using PCA analysis (Fig.3). In the PCA

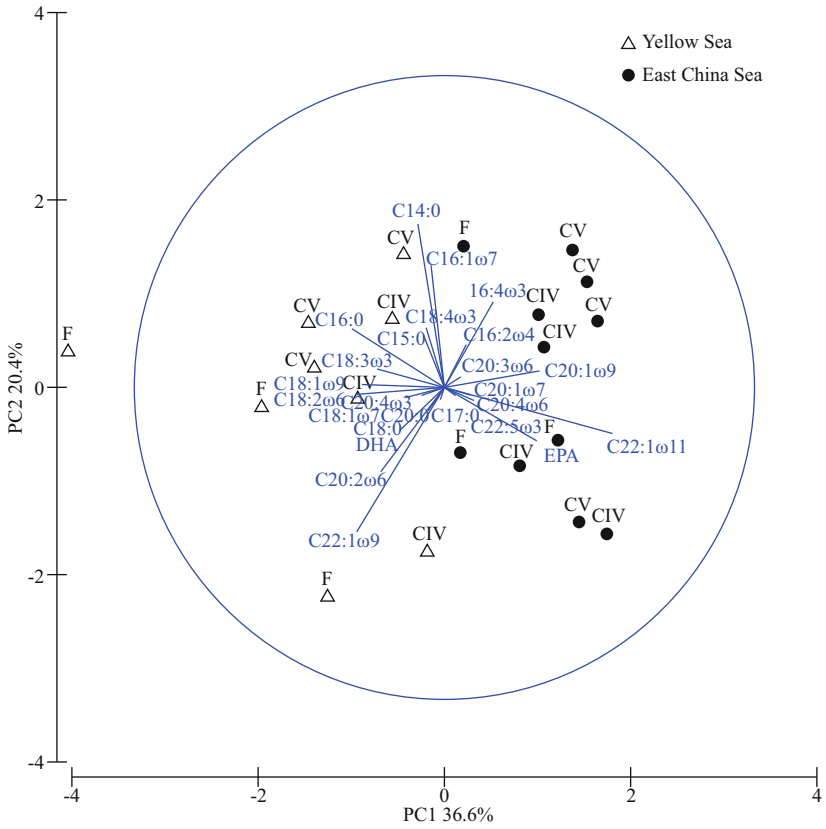

Fig.3 Principal component analysis (PCA) of *C. sinicus* samples based on their relative fatty acid compositions in Table 2

F: female; CV: copepodite stage V; CIV: copepodite stage IV.

plot, the first two principal components (PC1 and PC2) accounted for 36.6% and 20.4% of the total fatty acid variations, respectively. Some kinds of fatty acid, such as EPA, C20:1ω9, and C22:1ω11, were more abundant in the ECS, while some fatty acids, including C22:1ω9, 18:1ω9, and DHA, were higher in the YS (Table 2, one-way ANOVA, $P<0.01$). The results of SIMPER analysis (Table 4) showed the main contributors (such as C22:1ω11, EPA, DHA, and C20:1ω9), which lead to the fatty acid variations between the YS and the ECS individuals. These fatty acids accounted for over 50% of total fatty acid composition.

3.4 Factors affecting lipid accumulation

Environmental conditions differed between the YS and the ECS stations. The average water temperatures were 13.3 °C in the ECS and 9.1 °C in the YS during the sampling period though there were no differences in salinity (Table 5). The food conditions also differed between the two areas. Phytoplankton cell numbers in the ECS were significantly lower than in the YS while ciliate abundance shown a higher level in ECS. However, the differences of ciliate abundance and Chl-*a* concentrations were not significant between the ECS and the YS (Table 5).

BIO-ENV analysis was employed to identify single or combinations of environmental factors which contributed most to the variations in lipid and fatty acid compositions. The results showed that the temperature/salinity group attained the highest group score followed by temperature and temperature/salinity/ciliates group (Table 6).

4 DISCUSSION

4.1 Lipid content

Lipid profiles differed between both developmental stages and regions (the YS and the ECS). Generally, *Calanus* begin to synthesize wax esters at the CIII stage, reaching highest levels of lipid content at the CV stage (Kattner and Krause, 1987; Lee et al., 2006). As copepodid develops into an adult (or enters diapause in deep water), stored lipids are usually depleted gradually for reproductive or energy needs, resulting in the decline of stored lipids (Irigoien, 2004; Lee et al., 2006; Mayor et al., 2009). This leads to differences in lipids profiles among development

Table 4 Results of SIMPER analysis based on the fatty acid information in Table 2

	Dissimilarity (%)	Fatty acids & contribution (%)
Results of SIMPER analysis between Yellow Sea and East China Sea	18.6	C22:1ω11 (19.7%), C22:1ω9 (7.8%), C20:1ω9 (6.8%), EPA (6.6%), C20:2ω6 (5.6%), C14:0 (5.6%), 16:4ω3 (5.1%), C16:0 (5%), C16:1ω7 (4.8%), DHA (4.5%), C18:4ω3 (4.4%), C18:1ω9 (4.4%)

Fatty acids that contributed to top 70% of the dissimilarity of samples grouped by the Yellow Sea and the East China Sea are given.

Table 5 Environmental conditions and food availability in the Yellow Sea and the East China Sea

Variable		Yellow Sea	East China Sea	ANOVA (*P* value)
Food resource	Phytoplankton abundance ($\times10^6$ inds./m^3)	11.5±7.3	0.7±0.5	0.00**
	Chl *a* (mg/m^3)	3.7±1.5	3.1±0.9	–
	Ciliates ($\times10^3$ cells/L)	5.7±1.5	7.8±4.1	0.06
Temperature (℃)		9.1±0.5	13.3±0.7	0.00**
Salinity		32.4±0.5	31.6±0.7	0.12

– means no data; ** means significant difference.

stages of *C. sinicus* (Table 1). However, regional variation in lipid content of *C. sinicus* between the YS and the ECS may be influenced by internal and external factors, such as population state, food, temperature, and salinity conditions.

Population state is an internal factor which may influence lipids profiles of *Calanus*. Seasonal diapause *Calanus*, such as *C. finmarchicus*, usually accumulate a large amount of lipids in spring when the population recovers from the food limited winter (Lee et al., 2006). In this study, the high proportion of copepodite stage in the ECS indicates that *C. sincus* may be recovered earlier from winter population than in the YS. Earlier studies showed that hatching speed and development of nauplii and copepodites increased as temperature elevating (Uye, 1988). The higher temperature in the ECS might be the main reason to explain the earlier population recovery of *C. sincus* in the ECS. The *C. sincus* population in the YS and the ECS were clearly in different states. Though the population state is different, we compared the same development stages, from CIV stage to adult, to analyze the major external factors which may affect the lipid and fatty acid profiles.

For external factors, the BIO-ENV analysis showed that temperature played an important role in determining lipid content. Temperature can affect metabolic rates (Arts et al., 1993; Gillooly et al., 2001) and therefore influence the lipid usage (e.g. during a period of resource limitation or fasting) or restore (during refeeding). In this study, reginal differences were mainly identified in wax esters and triglycerides. When food is abundant, *Calanus* is capable of storing large amounts of wax esters. For example, *C. finmarchicus* has been reported to be able to store more than 20% of its body weight in wax esters (Diel and Tande, 1992). Stored wax esters are required during curial life-stage periods, such as dormancy or reproduction (Lee et al., 2006; Baumgartner et al., 2017). In this study, the food concentrations were high in both the YS and the ECS. However, the wax ester contents of *C. sinicus* were significantly low in the YS. This indicated that there are other factors, apart from food availability, influenced the wax ester contents. Previous studies have found that *C. sinicus* has an optimum temperature range of 10.0–20.0 °C (Huang and Zheng, 1986), and *C. sinicus* show a higher accumulation efficiency of lipids in 13.0 °C than 10.0 °C in laboratory incubation experiment (Zhou and Sun, 2017). In this study, water temperature in the ECS was more suitable for *C. sinicus*. And it might help *C. sinicus* to accumulate a higher level of storage lipid.

Table 6 Results of BIO-ENV analysis comparing biological data shown in Tables 1 & 2 and external condition data shown in Table 5

K	Spearman's rank correlation	Factor
1	0.61	Temperature
2	0.67	Temperature/salinity
2	0.57	Temperature/ciliates
2	0.47	Temperature/phytoplankton abundance
2	0.46	Ciliates/phytoplankton abundance
3	0.58	Temperature/salinity/ciliates
4	0.48	Temperature/Chl *a*/ciliates/phytoplankton abundance
5	0.45	All

K means number of factors.

Unlike wax esters, triglyceride is indicative of short-term feeding (Lee et al., 2006). Previous studies have shown that triacylglycerols are utilized more rapidly than wax esters in zooplankton and the fatty acids of triacylglycerol may be more reflective of recent food, whereas wax ester fatty acids and alcohols reflect both dietary influences and de novo synthesis (Lee et al., 2006). Because of the Assimilation of dietary fatty acids and de novo biosynthesis are rapid processes (Graeve et al., 2005), higher triglyceride content could indicate a more favorable feeding state. In this study, the triglyceride content shows a higher level in the ECS than in the YS. This indicates that the feeding environments are more favorable for *C. sinicus* in the ECS in comparison to the YS.

4.2 Feeding difference

In this study, wax ester fatty acid profiles (percentage composition) differed significantly between the YS and the ECS individuals. The main fatty acid classes of *C. sinicus* were similar to those in polar/subpolar *Calanus* sp. (such as *C. finmarchicus*). The main fatty acids recorded in *C. sinicus* were C14:0, C16:0, C16:1ω7, C18:1ω9, C18:4ω3, C20:1ω9, EPA, C22:1ω11, and DHA (Lee et al., 2006). It has been suggested that *Calanus* can incorporate unmodified dietary fatty acids into storage lipids (Lee et al., 1971), with fatty acid compositions varied with food availability. Therefore, variations in food conditions between the YS and the ECS stations might be partly responsible for the regional differences in fatty acid profiles.

SIMPER analysis results revealed that C22:1ω11, EPA, DHA, and C20:1ω9 were the top four contributors to the regional differences in fatty acid profiles. Previous studies (Napolitano, 1999; Dalsgaard et al., 2003) have reported that C22:1ω11 and C20:1ω9 are self-biosynthesized fatty acids of herbivorous *Calanus* species, while the DHA/EPA ratio can be used for trophic position comparison. In this study, the high levels of C22:1ω11, C20:1ω9 and low DHA/EPA ratio of *C. sinicus* in the ECS indicate that *C. sinicus* are feeding mainly on phytoplankton in this location. Though field studies have reported that *C. sinicus* was an omnivorous feeding species (Sun et al., 2006), it still feeds mainly on phytoplankton. Although grazing data was not analyzed in this study, it seems that *C. sinicus* in the YS feeds more non-phytoplankton food sources than in the ECS, such as ciliates, and exhibits higher C18:1ω9/∑herb (∑herb: the sum of 16:1ω7, 18:4ω3, and 18:1ω7) and DHA/EPA ratios. BIO-ENV analysis also showed that ciliates were important biological factors influencing the fatty acids profiles of *C. sinicus* though it does not show significant differences of Chl-*a* concentration and ciliate abundance between the ECS and the YS. Sun et al. (2006) found that *C. sinicus* feeds differently in different seasons and omnivorous feeding played a more important role for *C. sinicus* from summer to autumn. In this study, the environment was different between the ECS and the YS. Then *C. sinicus* may show different feeding selectivity in these two regions. In addition, this finally leads to the differences in fatty acids profiles of *C. sinicus*.

5 CONCLUSION

The spring phytoplankton bloom is an important period for coastal marine ecosystems. Copepods can store high levels of lipids during bloom periods. The results from this study indicate that temperature and nutritional variation directly influence lipid and fatty acid profiles in *C. sinicus*. This is the case for many polar *Calanus* species. Temperature may be the limiting external factor affecting lipid storage in *C. sinicus*, while regional food differences may affect fatty acid composition profiles of *C. sinicus*.

6 DATA AVAILABILITY STATEMENT

The data used in the current study are available from the corresponding author on reasonable request.

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